ASSESSMENT AND PREDICTION OF POTENTIALLY MINERALIZABLE ORGANIC NITROGEN FOR SUBARCTIC ALASKA SOILS

A

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ASSESSMENT AND PREDICTION OF POTENTIALLY MINERALIZABLE ORGANIC NITROGEN FOR SUBARCTIC ALASKA SOILS

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ABSTRACT

This study was to identify suitable methods to predict potentially mineralizable organic N for subarctic Alaska soils. Soil samples were taken from major agricultural area of subarctic Alaska. Laboratory incubation followed by kinetic model fit was first used to select a best model to estimate potential soil N mineralization. By correlating the model estimated organic N pool sizes and different chemical extracted organic N, I then found the best chemical method to estimate soil potentially mineralizable N. Spectroscopic properties of water extractable organic matter were also determined and correlated with model estimated organic N pool sizes in order to improve the estimation of soil mineralizable N pool. Finally, the best chemical method and spectroscopic property were used in the selected best kinetic model for the prediction of soil N mineralization in field incubation.

Model comparisons showed that models with fixed rate constants were better than that the ones with rate constants estimated from simulation. Among models with fixed rate constants, fixed double exponential model was best. This model differentiated active mineralizable organic N pool with a fixed rate constant of 0.693 $week^{-1}$ and slow mineralizable organic N pool with a fixed rate constant of 0.051 $week^{-1}$.

By correlating model estimated organic N pool size and chemical extracted organic N amount, I found that the potentially mineralizable organic N size was closely correlated with hot (80 °C) water extractable organic N or 1 *M* NaOH hydrolysable organic N. By correlating model estimated organic N size and spectroscopic characteristics of water extractable organic matter, I found that the active mineralizable

organic N pool was correlated with humification index in cold (22 °C) water extraction (R^2=0.89, p<0.05), which indicates that characterizing extracted organic matter was a useful tool to improve the estimation of soil organic N pools.

In summary, potential mineralizable organic N in soils from subarctic Alaska can be estimated by hot water extractable organic matter or 1 *M* NaOH hydrolysable organic N, which accounted for 70% and 63% of the variation in potentially mineralizable organic N, respectively. This approach will provide fundamental insight for farmers to manage N fertilizer application in agricultural land and also provide some basic information for ecologists on predicting N release from Alaska soil that can be used for assessing the N impact on ecosystem.

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CHAPTER 1 INTRODUCTION

1.1 Soil mineralizable N

Nitrogen is a key element for plant nutrient and growth. It is taken up mostly in inorganic forms by most plant species. In most soils, 95%-99% of the total nitrogen is in soil organic matter (Brady and Weil, 2008). The process in which organic nitrogen is transformed to ammonium (NH_4^+) by soil microorganisms is called mineralization. Nitrogen mineralization rates in soils can be affected by environmental conditions (such as soil moisture and temperature), soil properties (such as soil texture), and chemical nature of the soil organic matter. Most organic N mineralization can mostly occur within a temperature range of 5 to 35 °C, also can occur at temperature less than 0 °C. The rate of mineralization increases with the increase of temperature within this range (Stanford et al., 1972). The Q_{10} function, describing temperature response of soil N mineralization rate, decreases from 2.06 to 1.88 as temperature increases from 5 to 35 °C (Dessureault-Rompré et al., 2010), indicating that the response of mineralization rate is more sensitive to the increase of temperature in the low temperature range central. Alaska climatic conditions are characterized of long winter and relatively low average air temperature (average 17 °C in the summer of interior Alaska) (Wikipedia, 2011). An increase of 1.5 to 4.5 °C by 2050 in temperature of around arctic regions has been forecast (Coulson et al., 1996; Birks et al., 2004; Grogan and Jonasson, 2005). Such an increase of temperature in the cold Alaska regions may promote the activity of soil microorganisms and hence increase the mineralization of soil organic components. Alaska soils contain large quantities of soil organic matter that has accumulated (Billings, 1987; Hobbie et al.,

2000). As temperature increases, the induced increase of mineralized N from soil may be significant. The mineralized N in soil, if not used by plants, may result in a negative influence on the environment, such through ground and surface water pollution by leaching, and run off. Therefore, it is useful to be able to predict soil mineralizable N for crop production and ecological management because of the following reasons:

First, the accurate estimate soil mineralizable N is important for agriculture. Quantitative estimating potential mineralizable N can improve management practices that enhance plant N use efficiency and reduce over-use of fertilizer N in soils, resulting in reduction of N loss through NO_3^--N leaching, run-off, or volatilization. Also, reducing the rate of N use saves energy that is used for manufacturing fertilizers and reduces costs to farmers. In addition, over-use of N can result in more emission of greenhouse gas (nitrous oxide, N_2O) from nitrification and denitrification process. So, improving the N fertilizer use is likely to reduce these gas emissions.

Second, the accurate estimate of soil mineralizable N may be essential for understanding natural ecosystems. The increase of temperature will enhance rate of N release from soils, high N could change plant community dynamics and compositions. For example, some species take up more N and out compete other species, thus becoming dominant species and hence change the biodiversity of the ecosystem. This in turn may affect the animals that depend on these plant communities. In addition, high N release from soils, if not all used by plants, may be last through runoff and leaching to the streams or lakes, which will affect water quality.

Better estimates of soil mineralizble N can help us to develop a sound N management practice in soils to improve plant N use efficiency, and subsequently minimizing negative impacts of over use of N, also will understand and predict the trend of natural ecosystem in Alaska under warming climate conditions.

1.2 Existing methods to estimate soil mineralizable N

Traditionally methods used to estimate mineralizable N from soil organic matter are biological, physical, and chemical approaches.

Biological methods usually estimate mineralizable N by incubating a soil sample. The incubation can be conducted under aerobic or anaerobic condition. For laboratory incubation, soils usually are adjusted to water content conditions (such as 55-65% water holding capacity (WHC)) that are optimal or close to optimal for the mineralization process (Quemada and Cabrera, 1995; Gordillo and Cabrera, 1997). Incubation temperature typically ranges from 20, 25, 30, to 35 ^{o}C (Christensen and Olesen, 1998; Sharifi et al., 2007; Wang et al., 2001; Stanford and Smith, 1972). Incubation length also varies from 12 to 60 weeks (Gianello and Bremner, 1986; Bronson et al., 2001; Dendooven et al., 1997). Stanford and Smith (1972) suggested soils must be incubated for at least 24 week to obtain stable values of mineralizable N.

Biological approaches often assume that soil contains organic matter pools with different mineralization rates and these pools can be often estimated by fitting models on measured mineralized N data. Numerous kinetic models have been used to describe inorganic N production from soil or amended organic materials (Stanford et al., 1972; Juma et al., 1984; Molina et al., 1980). Soil N mineralization models are mainly

classified into simple exponential models, parallel models, and double exponential models (See Chapter 2). It is necessary to evaluate which model can better estimate the potential of Alaska soil' N mineralization.

Biological methods are considered to be the standard way to measure soil mineralizable N. However, the incubations are time-consuming (≥12 weeks) and labor intensive. Consequently, many researches have focused on the development of more rapid chemical or physical methods.

Chemical methods distinguish soil organic matter pools based on their respective solubility in various extraction chemicals (Coŭteaux et al., 2003; Kalbitz et al., 2003). Chemically extracted soil organic matter is expected to have different bioavailability of labile or recalcitrant fractions (Paul et al., 2006). In general, the labile fraction can have a large contribution to the estimation of potentially mineralizable organic N. Extraction chemicals include acid (eg. 1 *M* HCl), alkaline (eg. 1 *M* NaOH), and neutral solutions (eg. deionized water) (Hayes, 2006). In theory, the mild extractants (deionized water) are superior to harsh chemicals (acid, alkaline) because mild extractants obtain a smaller amount of non-labile N than the harsh chemicals that can mimic the amount of plant N uptake (Curtin et al., 2006). Recently, Curtin et al. (2006) evaluated the suitability of cold (22 °C) and hot (80 °C) water extractable N as predictors of soil N mineralization potential compared with indicators of total N, anaerobically and aerobically mineralizable N, and hot KCl extractable N. However, besides the chemical methods reported by Curtin et al. (2006), the commonly used chemical extractants also include 0.01 *M* $CaCl_2$ (Sharifi et al., 2007), ultraviolet (UV) absorbance at 205 and 260 nm of

0.01 *M* $NaHCO_3$ extraction, or others (Fox and Piekielek, 1978; Hong et al., 1990; Serna and Pomares, 1992). To the best of my knowledge, little information is available on the performance of cold and hot water extractable N as indices of potentially mineralizable N in soil compared with more widely used chemical methods. There is little current research about whether cold and hot water extractable organic N is better than acid or alkaline hydrolysable organic N in predicting soil potentially mineralizable organic N.

Water extractable organic matter (WEOM) in soil represents dissolved organic matter (DOM) which is a precursor of soil mineralization (Chantigny et al., 2010). WEOM is a mixture of organic matter with different bioavailabilities and not all WEOM is equally susceptible to biological decay (Bossier and Fontvieille, 1993; Boyer and Groffman, 1996). The relatively recalcitrant organic fractions contribute less to the potentially mineralizable N than the labile organic matter in WEOM. Determining the labile organic fraction in WEOM (eg. labile C and labile N ratio of WEOM) may help improving the estimation of soil N mineralization. However, due to the complex structure of soil organic matter, labile organic fractions that have greatest contribution to the mineralizable N during growing season are difficult to separate from recalcitrant organic fractions in WEOM. A simple method to differentiate labile from recalcitrant WEOM is fluorescence 3D spectroscopy, which has been successfully used to identify chemical characteristics and bioavailability of organic matter in seawater and forest soils (Yamashita and Tanoue, 2004; Fellman et al., 2008). The indices such as humification index (HIX), fluorescence efficiency (F_{eff}) calculated from fluorescence spectroscopies are correlated with change of riverine dissolved organic matter or WEOM (Wilson and

Xenopoulos, 2009) and the biodegradation of hot water extractable organic matter (Bu et al., 2010). The UV absorbance at 254 nm or 280 nm also indicate the aromaticity of organic matter (Kalbitz et al., 2003; Chin et al., 1994), which can be negatively correlated with biodegradability of organic matter. To characterize water extractable organic matter with fluorescence and UV spectroscopy might be helpful in improving the estimation of soil mineralizable N.

In addition to chemical methods, physical methods may also provide a quick estimation of mineralizable N. Physical methods separate soil constituents on the basis of density or size. Density fractionation uses a dense liquid such as $ZnBr_2$ solution (1.6 g cm^{-3} specific gravity) to separate a light fraction from a heavy fraction (Gregorich and Janzen, 1996; Wang et al., 2001). Light fraction N has been correlated with mineralizable N (Janzen, 1987; Curtin and Wen, 1999). However, light fractions also are reported to have little contribution to mineralizable N in soil (Boone, 1994; Sollins et al., 1984; Monaghan and Barraclough, 1995). Other physical methods include sieving soils into different particle size fractions and aggregate classes by ultrasonic vibration (Christensen, 1992; Six et al., 2004). Particulate organic matter isolated by dispersion and sieving of sand-size organic matter is considered labile (Cambardella and Elliott, 1992). Macroaggregates are demonstrated to include significant amounts of labile soil organic matter (Elliott, 1986; Gregorich et al., 1989). Physical methods consider arrangement of soil organomineral complexes, which may affect accessibility of mineralizable organic matter. However, interpretation of data derived from particle-size fractionation is difficult because the fractionation procedure of ultrasonic or other treatment may cause

transfer among particle size fractions (McGill et al., 1975). Therefore, physical methods were not selected for our study.

1.3 Assumption and objectives

This study assumed that: 1) soil N mineralization curves at 15 °C can be described and fitted by widely used kinetic models. A best model that can be used to predict soil N mineralization can be selected based on statistic methods; 2) a chemical method that is suitable for Alaska conditions can be selected by correlating the model estimated potentially mineralizable N sizes and widely used chemical methods; 3) the UV and fluorescence characteristics of water extractable organic matter are closely correlated with its biodegradability; and 4) chemical method and spectroscopic characteristics of WEOM can be used to estimate soil mineralizable organic N. Using the estimated potentially mineralizable organic N and best kinetic model can predict soil N dynamics in the field.

The objectives of this research were: 1) to estimate soil potentially mineralizable N by assessing kinetic models; 2) to estimate potentially mineralizable N suitable for Alaska soil by evaluating chemical methods; 3) to develop relationships between properties of water extracted organic matter (UV, fluorescence spectroscopy) with soil N pool biodegradability; and 4) to develop a method to predict N mineralization in the field using chemical methods and modified kinetic models obtained from laboratory incubation.

1.4 Thesis organization

This thesis consists of six chapters. Chapter 1 introduces the concept of mineralizable N, reviews current methods of estimating potentially mineralizable N, and proposes assumptions and objectives of this study (Fig. 1-1). The details of the study are covered by the next four chapters from Chapter 2 to 5. Chapter 2 described the N mineralization curves and selected models with statistical and biological meanings for estimating mineralizable N in subarctic soils. Soil samples with 55% water holding capacity were incubated under 15 °C for 24 weeks. Soil N mineralization curves were obtained by determining inorganic N contents periodically during the incubation. Based on curve shapes of N mineralization and proper statistic methods (Akaike's information criterion), best models for Alaska soils were selected among kinetic models (simple exponential, double exponential, parallel models, etc.) by fitting these models on inorganic N.

Chapter 3 follows with selections of chemical methods that can be used to estimate mineralizable N in subarctic Alaska soils. The best chemical method was selected from the widely used chemical methods by their correlations with the mineralizable N sizes estimated from best kinetic model selected in Chapter 2. Chemical methods included de-ionized (DI) water (22, 80 °C), UV absorbance at 260 and 205 nm of 0.01 *M* $NaHCO_3$, total C and N in soils, 1 *M* HCl, 1 *M* NaOH, and microbial biomass C and N. Best chemical methods for estimation of mineralizable N were those that were closely correlated with N_a, N_s, or N_0 representing fast, slow or potentially mineralizable N, estimated from best models selected from Chapter 2.

In Chapter 4, a study of spectroscopic characteristics of cold water (22 °C) and hot water (80 °C) extractable organic matter and their biodegradability is described. The biodegradability of extracted solutions was evaluated by the loss of water extractable organic C and N during a 21 d incubation. The UV and fluorescence spectrometers were used to characterize extracted solutions. Humification index, fluorescence efficiency, and fluorescence index were calculated using the data drawn from fluorescence spectroscopy.

In Chapter 5, mineralizable N pool sizes estimated by kinetic models were correlated with chemical methods and chemical characteristics of water extractable organic matter to investigate if prediction of potentially mineralizable N can be improved by characterizing water extractable organic matter. Another objective of this chapter was to test if chemically and model estimated potentially mineralizable N can be used to predict dynamics of N mineralization in the field. Mineralization rates in kinetic models were adjusted using data from a lab incubation under different temperature (5, 15, 25, and 35 °C) and soil moisture (20%, 55%, and 90% water holding capacity). The linear equation describing the correlation between models estimated potentially mineralizable N with chemically determined organic N was used to estimate mineralizable N for a specific soil. The best kinetic model selected from Chapter 2 was used to simulate the dynamic changes of N mineralization in the field. In order to avoid the influence of factors such as drying-rewetting cycles on soil N mineralization, field incubation with constant soil moisture (55% water holding capacity) was set up to check the feasibility of kinetic models to predict the N mineralization dynamics.

In Chapter 6, the thesis is synthesized. Contributions of this thesis to N mineralization estimations are summarized. In addition, several suggestions are made for future studies on the N mineralization estimations.

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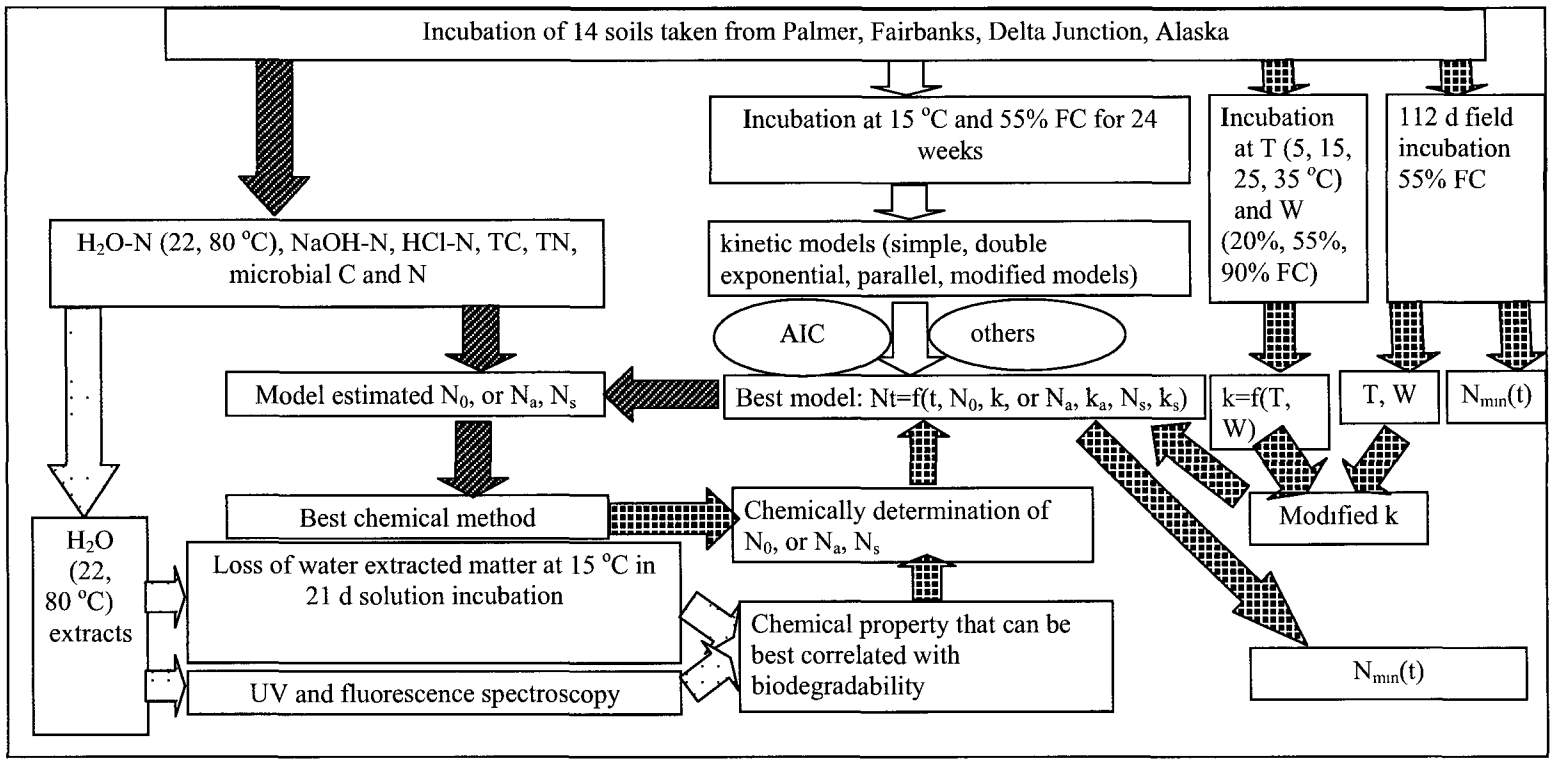

Fig. 1-1. Study outline for assessment and prediction of N mineralization in subarctic Alaska soils.
, experimental flow for objective 1 to predict potentially mineralizable N by evaluating kinetic models.
, experimental flow for objective 2 to predict mineralizable N by assessing chemical methods.
, experimental flow for objective 3 to characterize water extractable organic matter.
, experimental flow for objective 4 to predict mineralizable N in the field.
FC, water holding capacity. H_2O-N, water extractable organic N. NaOH-N, 1 *M* NaOH hydrolysable organic N. HCl-N, 1 *M* HCl hydrolysable organic N. TC, total carbon. TN, total nitrogen. $N_{min}(t)$, mineralized N at time t. N_0 and k, the organic N pool and its rate constant of simple exponential model. N_a, k_a, N_s, k_s, the organic activeand slow pool and their respective rate constants of double exponential model. y = f(x), y as a function of x. UV, ultra-violet spectroscopy.

CHAPTER 2 THE EVALUTION OF KINETIC MODELS OF N MINERALIZATION FOR SUBARCTIC ALASKA SOILS[1]

Abstract

Estimating soil mineralizable N is important for understanding soil N dynamics and improving N fertilization management strategies in soils. The estimation of soil mineralizable N varies depending on the model used. For this study, two groups of models were chosen. The first group contained nonfixed rate constants and unknown pool size. The second group contained fixed models with unknown pool size but fixed rate constants by values available from the literature. The objective of this study was to determine the best model among nonfixed or fixed models to predict mineralizable organic N in soils. Soils were incubated at 15 °C and 55% water holding capacity for 24 weeks in the laboratory. Inorganic N (NH_4^+-N + NO_3^--N) was determined at each sampling time. Different kinetic models of N mineralization were evaluated for three criteria including goodness of fit, the ability to obtain realistic parameter estimations, and effects of incubation time on model estimation (time sensitivity). Based on goodness of fit and time sensitivity, the nonfixed rate constant double exponential model was best among nonfixed models and the fixed double exponential model was best among fixed models. However, based on parameter reliability, the nonfixed double exponential likely produced unrealistic parameter estimations. Hence, the nonfixed simple exponential model was the best among nonfixed models. The fixed double exponential model was the best among fixed models.

[1] Zhao, A., Zhang, M., Valentine, D. 2011. The evaluation of kinetic models of N mineralization for subarctic Alaska soils. Prepared for submission in Soil Science.

2.1 Introduction

How much soil N can be made available for plant uptake during a growing season? This question drives a great deal of research into N availability, both to address fundamental questions of soil N dynamics and to improve recommendations for N fertilization rates. In most soils, 95% - 99% of the total nitrogen is in organic form in soils (Brady and Weil, 2008). These organic forms of N become available through the mineralization by soil microbes. In theory, given a soil, the pool sizes of these mineralizable organic N are soil specific and their mineralization rate constants vary with environmental conditions (Wang et al., 2004).

The estimation of mineralizable organic N pools is often based on measurement of the cumulative inorganic N released from soil during a laboratory incubation. The amount of inorganic N released during incubation depends on incubation time, soil moisture, and temperature. Incubation length in past studies has varied from 12 to 60 week (Gianello and Bremner, 1986; Bronson et al., 2001; Dendooven et al., 1997). Based on literatures, the optimum soil moisture for net N production normally falls at 55-70% water holding capacity (WHC) (Stanford and Smith, 1972; Linn and Doran 1984; Doran et al., 1990) or between -30 to -10 kPa of water tension (Stanford and Epstein 1974; MacDuff and White, 1985). Excessive soil moisture can increase the chance of N loss through denitrification and the low soil moisture can limit the microbial activity. Soil incubation temperature usually varies from 20 to 35 ^{o}C to mimic field condition or optimize microbe activity (Christensen and Olesen, 1998; Sharifi et al., 2007; Wang et al, 2001; Stanford and Smith, 1972). Soil incubation with a comparable temperature to field

condition seems reasonable because a high soil temperature such as 35 °C may rarely occur in cold areas such as Alaska. The rate of N mineralization exponentially increases with temperature within 5-35 °C (Stanford et al., 1972), which follows a function of

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)} \quad (2.1),$$

where Q_{10} is temperature coefficient, k_2 and k_1 are mineralization rate constants at different temperatures T_2 and T_1. Campbell et al. (1981) suggested that Q_{10}=2 can approximately estimate the response of N mineralization on temperature in most soils.

Cumulative inorganic N released from laboratory incubation is usually used for model fitting in order to estimate the rate of N mineralization and pool size of mineralizable N. Some widely used models were shown in Table 2-1. These models included two groups. Group 1 (unfixed model) contained traditional models with parameters of pool size and rate constant. These models are kinetic models of no biological meaning. Their parameter estimators change with incubation time length (Dou et al., 1996; Wang et al., 2003; Sierra, 1990). Group 2 (fixed model) contained models with unknown pool sizes but fixed rate constant (details shown later). The fixed rate constant models of this group were an improvement over unfixed rate constant models. By fixing the rate constant, the fixed models can eliminate the effect of incubation time on parameter estimation and produce the pool size that is independent on the incubation time. Hence, the fixed models are of biological meaning because they follow a theory that is used by Wang et al. (2003; 2004).

There were three widely used unfixed rate constant models including unfixed rate constant simple exponential model, unfixed double exponential model and unfixed

parallel model (Table 2-1). The unfixed simple exponential model (M1) assumes there is only one pool of soil mineralizable N (Stanford and Smith, 1972). The pool size of this model is N_0 (mg kg^{-1}) and the rate constant of N mineralization from the pool is k (wk^{-1}). The unfixed double exponential model (M2) assumes there are two mineralizable N pools in soil: an active pool (N_a, mg kg^{-1}) and a slow pool N_s (mg kg^{-1}). The rate constants of N mineralization are k_a (wk^{-1}) and k_s (wk^{-1}), respectively. Parallel model (M3) also assumes there are two (active and slow) pools (Bonde and Rosswall, 1987). The N mineralization from the active pool Na (mg kg^{-1}) follows an exponential curve but that from the slow pool is a linear one. The rate constant of N mineralization from this active pool is k_a (wk^{-1}). The rate constant of N mineralization from the slow pool is K (mg kg^{-1} wk^{-1}).

These unfixed models were fixed with the procedure used by Wang et al. (2003; 2004). Stanford and Smith (1972) took 39 soil samples across the USA, incubated these soils at 35 °C, and fitted the nonfixed simple exponential model to N mineralization data. They found that the k value of these soils averages 0.054 wk^{-1} and shows a small variation (0.009 wk^{-1}) across these soils. So, this average of 0.054 wk^{-1} is assumed to approximate the k values of unfixed simple exponential models in soils incubated at 35 °C. Using equation 2.1 and assuming Q_{10}=2, this value can be converted into 0.051 wk^{-1} at 15 °C. For a long incubation time, I assume that k value is mostly contributed by the N mineralization of the slow pool. Then, the 0.051 wk^{-1} can be used to replace k in simple exponential model and the k_s in double exponential model (Table 2-1). In double exponential and parallel models, the k_a is fixed by three different methods. In the first

method, the k_a is fixed based on the N mineralization curve (details shown in 2.3.1). In the second method, the k_a is replaced by the value 0.693 wk^{-1} that is used by Wang et al. (2004). They assume the half life of active pool N_a, which is responsible for the initial flush of N mineralization can be defined as 1 week. The rapid N mineralization during this short time period is mostly contributed by the active pools N_a and little by the slow pools N_s. During the short period of N initial flush, the N mineralization follows an exponential model of $1/2N_a=N_a\ e^{-k_a\ t_{1/2}}$. As such, $k_a=\ln 2/t_{1/2} = 0.693\ wk^{-1}$. In the third method, we adjusted the 0.693 wk^{-1} at 35 °C to 0.173 wk^{-1} at 15 °C by equation 2.1. The adjusted value of 0.173 wk^{-1} was used to replace the k_a in the double exponential model and parallel model.

All these nonfixed and fixed models are in nonlinear forms. They can be fitted at least by two approaches including least square and maximum likelihood. The nonlinear least square method is preferable to the maximum likelihood because the maximum likelihood is complex in its computation and it is used often for probability distributions rather than normal distribution (Benedetti and Sebastiani, 1996). For the least square method, nonlinear model fit is achieved only when the estimated parameters give minimal sum squares of the errors. Specifically, the nonlinear least square method can be conducted by different algorithms such as Gauss, Newton, Gauss-Newton, Levenberg–Marquardt. Levenberg-Marquardt is widely used for its adequate approach to find global converge (Wang et al., 2004). To obtain the parameter estimation using these algorithms, a set of initial values of unknown parameters is required. From the initial value(s), the model parameter(s) is calculated through one of the previously mentioned algorithms by

iterations. The iterations end (converge) when the difference of the sum squares in two iterations in a row changes less than a given value (in most software, 10^{-6}), and parameter(s) in the model is estimated. In addition, most software has set a limit for number of iterations. If the iteration exceeds this limit, a failure of converge will be made. Therefore, in nonlinear regressions, many problems exist, and these problems have persisted for many years (Seber and Wild, 2003). These problems stem from 1) selection of algorithms; 2) selection of initial values; 3) methods of iteration; 4) convergence conditions. Simulated parameters varies depending on different initial values, different iteration methods, and different converge conditions. One of solution to this problem is to try multiple sets of initial values. If the simulated parameters are the same for different initial values, the simulated parameters are reliable. In other words, the residue sum of squares might be a true minimal. However, the above mentioned problems will be even worse when more parameters need to be estimated in a model. Benbi and Richter (2001) found the inconsistent parameter estimators from double exponential models with four parameters based on different initial values. To avoid such problem, in this respect, a simple model with fewer parameters is preferred.

To select a suitable model among a set of models, a determination coefficient (R^2) is usually used for linear regression. However, R^2 alone can not be used as a criterion for nonlinear model selection because a model with more parameters yields a higher R^2 than a model with less parameters for a given set of data. It has to be adjusted with numbers of explanatory variables (so called adjusted determination coefficient, R^2_{adj}). The *F* test based on residual sum of squares is also used to compare nonlinear models (Hess and

Schmidt, 1995; Thuriès et al., 2001). But, *F* tests are restricted to model comparisons with nested relationships (Burnham and Anderson, 2003). For example, the simple exponential model is nested in the double exponential model but the parallel mode is not nested in the double exponential model. So, an *F* test can be used for comparison of simple and double exponential models but not for comparison of parallel and double exponential models. In statistics, nonlinear model comparisons often use the Akaike information criterion (AIC) or AIC adjusted for small sample sizes (AICc). These two criteria estimate the distance between a model and the "true" model that generate the data. The distance is the amount of information lost when using model to approximate the true model (Kullback and Leibler, 1951). The best model can be defined as the one which loses the minimum amount of information. They have been widely used for nonlinear model comparisons for other purposes (Saffron et al., 2006; Faour et al., 2007) but not for soil N mineralzation models. In addition, some studies use the standard errors of parameter estimates, or t-ratio (standard errors/parameter estimators) (Saviozzi et al., 1997; Thuriès et al., 2001) to indicate the reliability of estimated parameters. Sleutel et al. (2005) has found that a model may fit the data well, but the standard error of parameter estimators is large, which indicate a large variation in parameters including negative number or zero that is impossible in reality.

In summary, the estimation of soil mineralizable N depends on the model selection, and model evaluation methods. Therefore, the objectives of this study were to find the best model of predicting mineralizable N in subarctic soils by evaluating the performance of nonfixed and fixed soil N mineralization models based on the goodness of fit to

measured data, reliability of parameter estimation, and effect of incubation time on estimated parameters.

2.2 Materials and methods

2.2.1 Experimental sites and soil sampling

Fourteen soils were collected in major agricultural areas (Fairbanks, Delta Junction, and Palmer) of subarctic Alaska, USA. The soil series of these areas include Tanana (coarse-loamy, mixed, superactive, subgelic Typic Aquiturbels), Nenana (coarse-silty over sandy or sandy-skeletal, mixed, superactive Typic Haplocryepts), Volkmar (coarse-silty over sandy or sandy-skeletal, mixed superactive Aquic Haplocryepts), and Knik (coarse-silty over sandy or sandy-skeletal, mixed, superactive Typic Haplocryepts). Of the fourteen soil samples, nine were taken from the Delta Junction area. The soil samples (0-10 cm) were taken from three sites (three replicates for each site) in 2005. The other five soil samples were from the soil taken for other studies in 2007. Samples were taken from the 0-15 cm soil surface of agricultural land from Fairbanks Experiment Farm (two samples), Matanuska Experiment Farm near Palmer (one sample), Rosie Creek Farm near Fairbanks (one sample), and Delta Junction Field Research Site (one sample). All soil samples were air-dried and sieved (<2 mm) before beginning the experiments. Soil texture was analyzed by the hydrometer method after dispersion with sodium hexametaphosphate (Sheldrick and Wang, 1993). Soil pH was determined in a 1:1 soil/water suspension using a soil pH meter 140 (Corning Inc., New York, USA). Soil total C and N was measured by thermal combustion analysis using a LECO Truspec CN

analyzer (LECO Corporation., St. Joseph MI, USA). Table 2-2 gave results of soil characteristics including soil texture, pH, and total C and N concentrations.

2.2.2 Laboratory incubation

Each soil was incubated in triplicate in laboratory. Five grams of air dried sample were weighed into a 50 mL glass vial and wetted with distilled water to 55% water holding capacity. The vials were incubated for 24 weeks at 15 °C. During the incubation, the caps of glass vials were kept loose in order to keep the vial aerated. Soil moisture content was monitored and adjusted to 55% water holding capacity weekly. Soil samples were destructively collected at 0, 1, 2, 4, 6, 12, 16, and 24 weeks. Two grams of each sample were weighed out to determine moisture content by oven drying at 105 °C for 24 hours. One gram of each sample was weighed into a centrifuge tube and suspended in 10 mL 2 *M* KCl. The tube was shaken for 30 min on an Eberbach 6000 reciprocal shaker (Eberbach Corporation, Michigan, USA) at room temperature (22 ±1 °C). Then, the suspensions were filtered through about 2.5 μm filters (Whatman No.42 Ashless Filter, Whatman International Ltd, England). Inorganic N concentrations (NH_4^+-N + NO_3^--N) in extracts were determined using continuous flow analysis technique on an Alpkem Rapid Flow Analyzer (Astoria-Pacific International, Oregon, USA). All results were calculated based on the oven dry weight.

2.2.3 Soil N mineralization kinetic models

For all models, N_t was defined as the cumulative amount of N mineralized at time t and was calculated by subtracting inorganic N content (NH_4^+-N + NO_3^--N) at time zero

from that at the time of sampling. Table 2-1 showed the nonfixed and fixed models used in this study.

2.2.4 Data analysis

The N mineralization rate (mg kg^{-1} wk^{-1}) was calculated as inorganic N (NH_4^+-N + NO_3^--N, mg kg^{-1}) accumulated in a time period, that is, the N mineralization rate = (inorganic N at t_n - inorganic N at t_{n-1}) / (t_n-t_{n-1}). Differences between mineralization rates among sampling times were analyzed with one-way variance analysis (ANOVA), followed by least significance difference test (LSD) at probability of 0.01 and 0.05 levels.

The nonlinear regression of kinetic models was carried out with Matlab (Version 7.1, The MathWorks Inc., Massachusetts, USA). Estimators and standard error of parameters in nonlinear models were done with the widely used Levenberg–Marquardt algorithm and the iterative process.

I used adjusted R^2 (R^2_{adj}) to compensate for possible bias due to different number of parameters. The R^2_{adj} was calculated as:

$$R^2_{adj} = 1 - (n-1)(1-R^2)/(n-p),$$

where n=sample size, p=number of parameters, R^2 were produced by model simulation process. Similar with R^2, a high R^2_{adj} value indicates the model fit is better.

Nonlinear model selection was assessed by R^2_{adj} and AICc. The AICc was calculated as:

$$AICc = 2p + N \times (\ln 2\pi + 1 - \ln N + \ln SSE)) + 2p(p+1)/(N-p-1),$$

where p=number of parameters, N is sample sizes, and SSE is residue sum square. A small AICc value of models indicated a good simulation.

The reliability of estimated unknown parameters in all models was evaluated with t-ratio, which was

t-ratio = standard error of parameters / parameter estimators,

where standard error and estimators of parameters can be obtained from the model fit process. Robinson (1985) recommended an acceptable model to have standard error smaller than 50% of the estimated parameter.

2.3 Results

The soils taken from subarctic Alaska had loam or sandy loam texture (Table 2-2) These soils were agricultural soils with pH from 5.0-6.9, total C content from 2.3% to 5.7%, and C:N ratio from 15.4 to 24.0.

2.3.1 Soil N mineralization curves and model fit

The N mineralization followed exponential curve in most soils except for soil 10 (Fig. 2-1). The N mineralization curve of soil 10 displayed a long lag at the beginning of incubation. Visually, the N mineralization in different soils followed similar patterns: rapid mineralization at the beginning of incubation which then leveled off (Fig. 2-1).

By inspecting the N mineralization curves and N mineralization rate curves (Fig. 2-1), I found that the initial flush of N mineralization lasted for about 6 weeks in most soils. In the double exponential model and parallel model, the active pool Na is assigned to be responsible for this initial flush. In other words, the half life of active pool N_a can be defined as 3 weeks. I assumed that the slow pool of organic N contributes to little initial N flush during this short period. Then, during the initial N flush period, the N mineralization follows an exponential model of $1/2N_a=N_a\, e^{-k_a\, t_{1/2}}$. As such, $k_a=\ln 2/t_{1/2}$ =

0.231 wk^{-1}. Therefore, we used 0.231 wk^{-1} to approximately replace k_a in double exponential model and parallel model (Table 2-1).

2.3.2 Model fit to soil N mineralization

Soil N mineralization did not follow exponential curves in soil 10. So, we did not fit exponential model to this soil. The soil N mineralization curves were fitted using nonfixed and fixed exponential models. The R^2_{adj} values of nonfixed models all were above 0.98, indicating all nonfixed models can fit N mineralization well (Table 2-3). Among these nonfixed models, based on R^2_{adj} values, no single model consistently showed better fit than the others. The R^2_{adj} values had little difference among models for each soil. For example, the R^2_{adj} values were of minor (0.0001) difference among the nonfixed double exponential and parallel model for the soil 13 and 14. AICc values showed that the nonfixed double exponential model was better than nonfixed parallel model, followed by nonfixed simple exponential model in most soils (Table 2-3).

For models fixed with k_a = 0.231 wk^{-1}, the fixed parallel model showed the smallest AICc values among the three fixed models in most soils (Table 2-4), indicating that the fixed parallel model might be better than the others. For models fixed with k_a = 0.693 wk^{-1}, the fixed double exponential model showed the smallest AICc values among the three fixed models in most soils, indicating that the fixed double exponential model might be better than the others. For models fixed with k_a = 0.173 wk^{-1}, the fixed parallel model showed the smallest AICc values among the three fixed models in most soils, indicating that the fixed parallel model might be better than the others.

2.3.3 The reliability of estimated parameters

For each soil, the parameters' reliability from different models was assessed with t-ratio. A large standard error or t-ratio that was larger than 50% may indicate that the true parameter value could be within a range including zero or negative values, which would be impossible in reality. Also, the t-ratio larger than 50% indicates wide uncertainty of parameter estimations. Among nonfixed models (Table 2-5), the t-ratio of estimated parameters from nonfixed double exponential model was larger than 50% in most soils, A t-ratio larger than 50% was also found in nonfixed parallel models for five out of thirteen soils. The larger t-ratio in these two nonfixed models indicated that the estimated parameters from nonfixed parallel and double exponential model were not reliable.

For models with k_a fixed at 0.231 wk^{-1}, the t-ratio was larger than 50% in estimated parameters from fixed parallel and double exponential models (Table 2-6). For models fixed with k_a=0.173 wk^{-1}, the t-ratio was larger than 50% in estimated parameters from fixed parallel and double exponential models. The larger t-ratio values of parameters from models fixed with k_a 0.231 and 0.173 wk^{-1} indicated that the fixed parallel model was not reliable to give realistic estimation of mineralizable N pool size. The models fixed with k_a 0.693 wk^{-1} showed t-ratios smaller than 50%, indicating the reliability of these fixed models.

2.3.4 Effect of incubation time on model estimation

Soil 3 was randomly selected to evaluate each model's sensitivity to change in incubation time. The model parameters were estimated from different models by different incubation times of 12, 16, and 24 weeks. Then, these parameters were used to

estimate inorganic N at each time point within 24 weeks. A good model should show less sensitivity to change in incubation time. A less time sensitive model should produce the relatively closer curve fit of estimated N mineralization among different duration incubation times. The far departure of estimated N mineralization curves from different incubation time indicates that the model is time sensitive. Figure 2-2 shows that the N mineralization curves estimated from unfixed double exponential model based on data from 12, 16 and 24 weeks incubation had little departure from each other than that from the unfixed simple exponential model, followed by unfixed parallel model. The fixed double exponential model was least time sensitive among fixed models (Fig. 2-3).

2.3.5 Estimated parameters

The model estimated mineralizable N potentials were different among nonfixed models for each soil (Table 2-7). The potentially mineralizable N pool (N_0) determined by nonfixed simple exponential models ranged from 14 to 87 mg N kg^{-1}. The total mineralizable N pool (N_a+N_s) of fixed double exponential model ranged from 30 to 132 mg N kg^{-1}. During a 24-week incubation, total inorganic N measured in the soils (N_{24wk}) ranged from 22 to 92 mg kg^{-1}. As an estimation of soil potentially mineralizable N, the N_a+N_s values were larger than that measured N_{24wk} for soils. However, the N_0 values were similar to the measured N_{24wk} for the most soils but slightly smaller for the 4 soils.

For models fixed with k_a 0.231 or 0.173 wk^{-1}, the estimated parameter C from parallel model was negative numbers in some soils (Table 2-8).

The estimated mineralizable N from nonfixed simple exponential model was linearly correlated with that estimated from double exponential model but little related

with that estimated from nonfixed double exponential model (Fig. 2-4). On average, the total potentially mineralizable N (N_a+N_s) from double exponential model fixed with k_a 0.693 wk^{-1} was 33% higher than that estimated from nonfixed simple exponential model (N_0).

2.4 Discussion

2.4.1 The evaluation criterion of nonlinear models

The goodness of fit of models are often compared based on MSE or residual variance based *F* test, or R^2_{adj} values which is R^2 adjusted with numbers of explanatory terms (Hess and Schmidt, 1995; Ellert and Bettany, 1988; Thuriès et al., 2001; Saviozzi et al., 1997). But, the *F* test is restricted only to nested models (Burnham and Anderson, 2003). Models here included non-nested models such as double exponential and parallel models so that the *F* test was not appropriate for this study. In some studies, R^2 is viewed as the percentage of variability explained by the nonlinear model (Thuriès et al., 2001). But, in nonlinear regression, the total sum of squares (SST) is not equal to the regression sum of squares (SSR) plus the residual sum-of-squares (SSE) as is the case in linear regression (Spiess and Neumeyer, 2010). It is inappropriate to interpret the determination coefficient as the percentage of variability explained by a nonlinear model when determination coefficient is calculated using an equation of 1-SSE/SST as in most software (eg. SAS PROC NLIN) rather than SSR/SST. In addition, it might be misleading to compare models with different parameter numbers if R^2 is not adjusted by numbers of explanatory terms because increasing parameter numbers in models always increase R^2 value. The R^2_{adj} penalizes by the number of terms (p + 1) in the model but it

is still a weak tool for nonlinear model comparisons. This study showed that R^2_{adj} did not consistently show which model is best among nonfixed models, indicating the R^2_{adj} is a weak tool for nonlinear model comparisons. We also found little difference in R^2_{adj} among models. This finding was not new. The small change (<0.001) in R^2_{adj} values is also presented by Ellert and Bettany (1988) and Sleutel et al. (2005). In these studies, R^2_{adj} values were same among some models (R^2_{adj} = 0.994) for N mineralization from wheat/fallow soils (Ellert and Bettany, 1988), and among single first-order, parallel first-order, and parallel first- and zero-order kinetic models (R^2_{adj} = 0.99789) for respiration data from soil amended with anaerobic compost (Sleutel et al., 2005). Undoubtedly, a small improvement or no change in R^2_{adj} was not helpful in determining which model can best fit data well. Spiess and Neumeyer (2010) even suggest not reporting it in nonlinear model regression and use AICc to replace it.

The AIC or AICc penalizes models with more parameters in model comparisons. In a simulation study investigating the power of AICc and R^2_{adj} to find a given true model, it is reported that the chance (80%) of AICc to find the true model is higher than that of R^2_{adj} (40%) (Spiess and Neumeyer, 2010). In this study, we found that AICc values among different models had a distinct difference, which make it easy to find a best model. Therefore, we suggest to use AICc for nonlinear N mineralization model comparison.

In a study of N mineralization kinetics, t-ratio was found to provide no consistent trend for various models (Benbi and Richter, 2001). However, in this study, a large t-ratio consistently occurred to nonfixd double exponential models and nonfixed parallel model. These two models contain more than three parameters, which may result in a

difficulty in finding optimal parameter estimator due to the complex computation for more parameters. Hess and Schmidt (1995) also reported a larger t-ratio in models with more parameters than simple model with less parameters. In this study, although the nonfixed double exponential model was best in fitting data based on AICc and time sensitivity, t-ratio showed that this model had a false reliability of parameter estimation. It strongly suggested that t-ratio is an important criteria for determining realistic parameter estimators if a goal of a study is to obtain these parameters, for example, in studying the effects of land use change on potentially soil mineralizable N pool.

2.4.2 Nonlinear model comparisons

In this study, soils from Delta Junction, Fairbanks, and Palmer areas of Alaska were incubated at 15 °C and 55% water holding capacity for a 24-week incubation. Thirteen of fourteen soils followed exponential curves and the remaining one displayed a long lag at the beginning of incubation. Exponential curves for N mineralization over time have been reported (Stanford and Smith, 1972; Wang et al., 2001). However, some also show a S shape (Houot et al., 1989; Ellert, 1990). The S-shaped type of curve has been attributed to immobilization induced by low temperature (Campbell et al., 1995). In this study, the exponential curves at low temperature incubation indicated that the gross mineralization was larger than gross immobilization in most soils. The soil N mineralization curves vary depending on substrate availability, microbial activity, or the balance of gross mineralization and immobilization (Ellert and Bettany, 1988).

The parameter values of k_a and k_s from nonfixed double exponential model were same for some soils (4 soils), indicating that nonfixed simple exponential might be best

for these soils. Based on the AICc and time sensitivity of models, we found that nonfixed double exponential model was best among nonfixed models. However, based on t-ratio, we found that nonfixed double exponential and nonfixed parallel model were likely to produce unreliable parameter estimation, indicating these two models were not reliable to estimate soil mineralizable organic N. So, we suggested the nonfixed simple exponential model as the best model to predict mineralizable N among the three nonfixed models. For the nonfixed double exponential model, the estimated parameters of k_s was small and N_s was large in four soils (Table 2-7), which may indicate that the incubation length of 24 week is still too short to have the N mineralization curve level off. Due to these four large N_s values, the N_a+N_s of nonfixed double exponential model showed a poor correlation (R^2=0.1363) with N_0 of nonfixed simple exponential (Fig. 2-4).

Three different k_a values were used to fix models here. Based on AIC values, t-ratios, and estimated parameters, we found that the models fixed with k_a 0.231 and 0.173 wk^{-1} were likely to produce unreliable or negative parameter estimators, indicating the models fixed with these two k_a values were not reliable. The models fixed with k_a 0.693 wk^{-1} were better in their parameter reliability than the models fixed with the other two methods. This might indicate that a half life of 1 week for active pool N_a used for soils incubated at 35 °C was also good for soils incubated under 15 °C. The similar half life of N_a might be reasonable. Studies have found that there is a flush of N release for the first two weeks (Stanford and Smith, 1972), which can be described by the double exponential model (Cabrera and Kissel, 1988). The N flush is attributed to the easily mineralizable N released from soil pretreatment such as air drying, sieving or others

(Benbi et al., 2002). It is likely that these easily mineralizable N can be rapidly used by microbes as long as the environment condition does not limit the microbe activity. In other words, within the temperature range that can not limit microbe activity, the mineralization of this easily mineralizable N is little influenced by temperature difference.

Among models fixed with k_a 0.693 wk^{-1}, fixed double exponential model was best in terms of AICc, time sensitivity, and t-ratio. The pool size of N_a+N_s from fixed double exponential models was highly correlated (R^2 = 0.9983) with the N_0 from nonfixed simple exponential models, indicating that the fixed double exponential can be used to predict potentially mineralizable organic N. From the nonfixed simple exponential model, we found that the size of estimated mineralizable N (N_0) was similar or slight smaller N_0 values than N_{24wk} (Table 2-7). Dou et al. (1996) also reported very close even smaller N_0 values than the measured inorganic N during 30 weeks incubation and suggested that simple exponential model is weak to fit data since N_0 is supposed to be larger than the measured values as an upper limit of potentially mineralizable N. In agreement with this conclusion, the N_a + N_s from fixed double exponential model but not N_0 from nonfixed simple exponential model here was considered as a reliable prediction of potentially mineralizable N.

2.5 Conclusions

Soil N mineralization in subarctic Alaska soils generally followed exponential curves. Different exponential models can fit these data. From a comprehensive consideration of statistical criterion (AIC, t-ratio) and theoretical meaning (time

sensitivity, parameter values), we concluded that double exponential model fixed with k_a 0.693 wk^{-1} and k_s 0.051 wk^{-1} was best to obtain an reliable estimation of parameters.

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Table 2-1. List of widely used kinetic models for estimating N mineralization in soils.

	Equation	Reference	Fixed model	Equation
Nonfixed Simple exponential (M1)	$N_t = N_0 (1-e^{-kt})$	Stanford and Smith (1972)	Fixed simple exponential (M1f)	$N_t = N_0 (1-e^{-0.051t})$
Nonfixed Double exponential (M2)	$N_t = N_a (1-e^{-k_a t}) + N_s (1-e^{-k_s t})$	Molina et al.(1980)	Fixed double exponential (M2f)	$N_t = N_a (1-e^{-k_a t}) + N_s (1-e^{-0.051t})$
Nonfixed Parallel (M3)	$N_t = N_a (1-e^{-k_a t}) + Kt$	Bonde and Rosswall (1987)	Fixed parallel (M3f)	$N_t = N_a (1-e^{-k_a t}) + Kt$

In the fixed double exponential and parallel model, k_a was replaced with 0.231 wk^{-1}, or 0.693 wk^{-1}, or 0.173 wk^{-1}.

Table 2-2. List of basic properties of Alaska soils.

Soil	Land use	Location	Texture	pH[1]	Total C kg kg^{-1}	Soil C:N
1	CRP[2]	Delta Junction	Sandy loam	5.4	0.045	22.5
2	Forest	Delta Junction	Sandy loam	5.2	0.047	23.5
3	Agriculture	Delta Junction	Sandy loam	5.4	0.057	19.0
4	CRP	Delta Junction	Sandy loam	5.4	0.041	20.5
5	Forest	Delta Junction	Loam	5.4	0.048	24.0
6	Agriculture	Delta Junction	Loam	5.4	0.038	19.0
7	Agriculture	Palmer	Loam	5.3	0.031	15.7
8	Agriculture	Delta Junction	Loam	5.1	0.033	19.4
9	Agriculture	Fairbanks	Loam	6.9	0.023	12.0
10	Agriculture	Fairbanks	Loam	6.2	0.029	15.4
11	Agriculture	Fairbanks	Sandy loam	6.7	0.036	18.1
12	CRP	Delta Junction	Loam	5.3	0.083	16.6
13	Agriculture	Delta Junction	Loam	5.0	0.050	16.7
14	Forest	Delta Junction	Loam	5.4	0.048	24.0

[1] Deionized water (solution:soil=1:1 (v:w)).
[2] CRP, conservation reserve program.

Table 2-3. Adjusted determination coefficient (R^2_{adj}) and Akaike information criterion (AICc) from different nonfixed models for each soil

	R^2_{adj}			AIC_c		
soil	M1	M2	M3	M1	M2	M3
1	0.9979	**0.9993**	0.9976	6.8	**-9.3**	5.1
2	0.9795	**0.9824**	0.9743	8.0	**3.8**	6.9
3	0.9966	**0.9997**	0.9988	13.0	**-10.7**	4.8
4	**0.9976**	0.9967	0.9953	7.3	**6.7**	9.5
5	0.9980	**0.9983**	0.9976	-12.0	**-16.3**	-13.4
6	0.9767	**0.9989**	0.9986	27.8	**-4.4**	2.6
7	0.9934	0.9979	**0.9984**	3.3	**-13.5**	-11.0
8	0.9765	0.9903	**0.9984**	13.7	-0.9	**-11.0**
9	0.9880	0.9945	**0.9951**	5.5	**-8.3**	-4.6
11	0.9766	0.9917	**0.9936**	13.4	**-2.4**	0.1
12	0.9752	**0.9995**	0.9967	25.5	**-12.8**	9.2
13	0.9784	0.9995	**0.9996**	24.6	-8.7	**-9.8**
14	0.9936	**0.9946**	0.9945	-12.0	**-16.3**	-13.3

Bold numbers indicated the highest R^2_{adj}, the lowest AICc among different models for each soil.
M1, simple exponential model: $N_t = N_0 (1-e^{-kt})$
M2, double exponential model: $N_t = N_a (1-e^{-k_a t}) + N_s (1-e^{-k_s t})$
M3, parallel model: $N_t = N_a (1-e^{-k_a t}) + Kt$

Table 2-4. Akaike information criterion values (AICc) from different models fixed with different k_a values for each soil.

soil	k_a=0.231 wk^{-1}			k_a=0.693 wk^{-1}			k_a=0.173 wk^{-1}		
	M1f	M2f	M3f	M1f	M2f	M3f	M1f	M2f	M3f
1	48.1	**21.2**	24.2	48.1	**38.6**	45.8	48.1	22.1	**21.2**
2	35.8	28.8	**21.4**	35.8	**32.6**	35.1	35.8	28.6	**22.1**
3	50.4	20.1	**20.0**	50.4	**37.4**	45.8	50.4	28.3	**28.2**
4	42.4	**28.3**	32.8	42.4	**38.7**	46.3	42.4	**24.0**	26.0
5	11.1	**9.0**	17.1	**11.1**	14.4	26.1	11.1	**6.8**	13.0
6	46.1	36.4	**32.3**	46.1	16.3	32.9	46.1	39.8	**38.3**
7	31.4	8.9	**4.2**	31.4	**20.4**	29.2	31.4	14.5	**12.5**
8	30.8	23.2	**19.5**	30.8	**23.5**	27.6	30.8	25.7	**24.0**
9	38.7	61.9	**22.0**	38.7	**18.2**	24.5	38.7	31.7	**28.0**
11	**37.2**	38.9	39.0	**37.2**	42.8	44.6	**37.2**	38.4	38.2
12	46.0	42.6	**38.0**	46.0	**20.2**	19.4	46.0	45.1	**41.7**
13	49.4	37.4	**36.4**	49.4	**14.5**	32.6	49.4	41.4	**41.2**
14	46.0	33.7	**30.2**	46.0	**22.3**	33.2	46.0	37.8	**37.1**

Bold numbers indicated the lowest AICc among different models for each soil.

M1f, fixed simple exponential model: $N_t = N_0 (1\text{-}e^{-0.051t})$

M2f, fixed double exponential model: $N_t = N_a (1\text{-}e^{-k_a t}) + N_s (1\text{-}e^{-0.051t})$

M3f, fixed parallel first and zero order kinetic model: $N_t = N_a (1\text{-}e^{-k_a t}) + Kt$

Table 2-5. The t-ratio (standard error/parameter estimator, expressed as %) for each parameter in different nonfixed models for each soil.

soil	M1		M2				M3		
	N_0	k	N_a	k_a	N_s	k_s	N_a	k_a	K
1	1	4	44	**222**	2	5	9	10	**210**
2	4	12	**T**	**T**	**T**	**T**	20	27	**T**
3	2	5	36	31	6	9	6	8	30
4	2	5	**90**	**T**	**T**	**T**	19	20	**T**
5	3	6	**T**	**T**	**T**	**T**	42	33	**T**
6	5	13	23	27	33	**66**	5	9	6
7	3	7	**65**	45	**991**	**T**	7	9	16
8	6	14	**139**	**116**	**T**	**T**	13	21	17
9	2	8	46	39	30	**62**	6	11	25
11	3	11	29	37	**T**	**T**	5	10	21
12	4	12	9	11	2	11	6	12	12
13	4	12	15	15	**74**	**109**	2	4	4
14	4	9	**T**	**T**	**T**	**T**	**59**	48	**T**

Bold numbers indicate the t-ratio larger than 50% for each parameter from different nonfixed models.

M1, nonfixed simple exponential model: $N_t = N_0 (1\text{-}e^{-kt})$

M2, nonfixed double exponential model: $N_t = N_a (1\text{-}e^{-k_a t}) + N_s (1\text{-}e^{-k_s t})$

M3, nonfixed parallel kinetic model: $N_t = N_a (1\text{-}e^{-k_a t}) + Kt$

T, The t-ratio is larger than a thousand.

Table 2-6. The t-ratio (standard error/parameter, expressed as %) of each parameter for model fixed with different k_a values.

soil	k_a=0.231 wk^{-1}					k_a=0.693 wk^{-1}					k_a=0.173 wk^{-1}				
	M1f	M2f		M3f		M1f	M2f		M3f		M1f	M2f		M3f	
	N_0	N_a	N_s	N_a	K	N_0	N_a	N_s	N_a	K	N_0	N_a	N_s	N_a	K
1	9	4	17	3	22	9	13	11	13	18	9	1	0	3	**121**
2	12	6	**0**	7	**134**	12	21	28	17	35	12	4	0	7	36
3	8	3	14	2	14	8	10	9	11	15	8	6	**T**	4	**339**
4	7	10	15	7	21	7	20	10	18	17	7	7	29	5	36
5	3	23	8	13	13	3	39	6	26	12	3	19	10	10	15
6	7	13	21	6	16	7	4	2	6	6	7	17	**58**	9	46
7	7	6	10	3	8	7	12	7	12	12	7	8	39	4	31
8	7	15	22	7	16	7	16	8	12	11	7	18	**53**	9	41
9	11	**335**	**T**	6	**T**	11	6	10	7	16	11	4	0	9	**54**
11	12	32	**187**	21	**231**	12	53	34	35	41	12	7	0	20	**235**
12	15	8	0	11	80	15	5	15	3	14	15	38	**T**	15	57
13	9	11	**67**	7	54	9	3	3	6	9	9	16	**T**	10	**332**
14	8	11	28	6	21	8	5	4	7	8	8	15	**148**	9	**98**

Bold numbers indicate the t-ratio larger than 50% for each parameter from different fixed models.

M1f, fixed simple exponential model: $N_t = N_0 (1-e^{-0.051t})$

M2f, fixed double exponential model: $N_t = N_a (1-e^{-k_a t}) + N_s (1-e^{-0.051t})$

M3f, fixed parallel first and zero order kinetic model: $N_t = N_a (1-e^{-k_a t}) + Kt$

T, t-ratio larger than 1000%

Table 2-7. The estimated mineralizable N values from each model for each soil.

Soil	M1		M2				M3			Incubation
	N_0	K	N_a	k_a	N_s	k_s	N_a	k_a	K	N_{24wk}
1	71.28	0.18	3.70	3.02	68.76	0.16	68.46	0.19	0.12	70.29
2	22.72	0.24	**9.07**	**0.24**	**13.93**	**0.24**	23.13	0.24	0.00	22.51
3	**82.75**	0.18	13.92	0.69	72.48	0.13	67.96	0.23	0.67	83.28
4	69.18	0.14	**7.86**	**0.15**	**59.89**	**0.15**	68.14	0.14	0.00	65.63
5	25.70	0.09	**7.34**	**0.09**	**18.35**	**0.09**	25.85	0.08	0.00	22.12
6	82.95	0.15	29.56	0.53	102.90	0.03	40.10	0.39	1.93	86.09
7	32.63	0.15	20.86	0.24	54.03	0.01	21.95	0.24	0.46	33.10
8	33.39	0.14	16.79	0.32	130.11	0.01	17.38	0.31	0.70	34.89
9	27.32	0.27	13.96	0.54	15.87	0.10	21.89	0.37	0.29	28.85
11	26.74	0.18	**9.13**	**0.18**	**17.61**	**0.18**	40.15	0.13	0.55	25.61
12	**31.92**	0.42	26.32	0.58	113.18	0.00	26.41	0.57	0.34	35.39
13	**67.80**	0.21	24.04	0.81	56.44	0.08	42.04	0.43	1.30	72.05
14	**68.45**	0.17	34.45	0.41	100.00	0.02	38.64	0.37	1.42	72.80

Bold values for model M1 indicates that values are smaller than that of N_{24wk} (mg kg^{-1}). Bold values for Model M2 indicates that there is same k_a and k_s values for soil.

M1, nonfixed simple exponential model: $N_t = N_0 (1\text{-}e^{-kt})$

M2, nonfixed double exponential model: $N_t = N_a (1\text{-}e^{-k_a t}) + N_s (1\text{-}e^{-k_s t})$

M3, nonfixed parallel kinetic model: $N_t = N_a (1\text{-}e^{-k_a t}) + Kt$

Table 2-8. The estimated mineralizable N values from models fixed with different k_a values for each soil.

soil	k_a=0.231 wk^{-1}					k_a=0.693 wk^{-1}					k_a=0.173 wk^{-1}				
	M1f	M2f		M3f		M1f	M2f		M3f		M1f	M2f		M3f	
	N_0	N_a	N_s	N_a	K	N_0	N_a	N_s	N_a	K	N_0	N_a	N_s	N_a	K
1	121.73	54.12	24.82	58.79	0.55	121.73	25.54	71.05	32.98	1.85	121.73	72.18	0.00	73.76	**-0.10**
2	40.89	24.09	10.00	24.09	**-0.08**	40.89	11.09	18.88	13.26	0.47	40.89	24.65	0.00	30.15	**-0.33**
3	141.89	62.88	29.29	67.97	0.67	141.89	30.17	82.00	38.56	2.16	141.89	83.97	0.00	84.97	**-0.05**
4	110.44	37.01	44.16	45.51	0.96	110.44	16.81	77.08	24.82	2.01	110.44	51.87	22.81	57.56	0.44
5	34.03	4.69	25.62	9.43	0.57	34.03	1.93	30.20	4.91	0.80	34.03	6.74	22.64	12.07	0.45
6	134.44	45.66	52.69	53.85	1.28	134.44	23.58	87.65	31.66	2.38	134.44	61.50	30.55	66.68	0.74
7	53.29	19.01	19.26	22.26	0.45	53.29	9.09	35.26	12.55	0.94	53.29	26.06	9.26	27.80	0.21
8	53.47	17.32	22.46	20.82	0.54	53.47	8.52	36.56	11.93	0.99	53.47	23.38	13.96	25.80	0.33
9	50.30	8.57	**0.00**	28.79	**-0.04**	50.30	14.75	21.02	16.87	0.56	50.30	30.25	0.00	35.66	**-0.33**
11	45.53	21.54	6.95	23.19	0.13	45.53	8.25	29.15	11.66	0.73	45.53	27.11	0.00	29.45	**-0.14**
12	62.11	30.30	10.00	40.74	**-0.35**	62.11	23.42	15.63	24.78	0.43	62.11	38.03	0.00	49.88	**-0.73**
13	119.88	56.99	17.83	59.18	0.47	119.88	29.10	62.13	35.01	1.67	119.88	71.02	0.00	73.24	**-0.12**
14	115.90	46.13	33.29	50.98	0.83	115.90	23.38	69.50	29.87	1.88	115.90	62.33	10.60	63.09	0.32

Bold numbers indicate negative values for estimated parameters from models.

M1f, fixed simple exponential model: $N_t = N_0 (1\text{-}e^{-0.051t})$

M2f, fixed double exponential model: $N_t = N_a (1\text{-}e^{-k_a t}) + N_s (1\text{-}e^{-0.051t})$

M3f, fixed parallel first and zero order kinetic model: $N_t = N_a (1\text{-}e^{-k_a t}) + Kt$

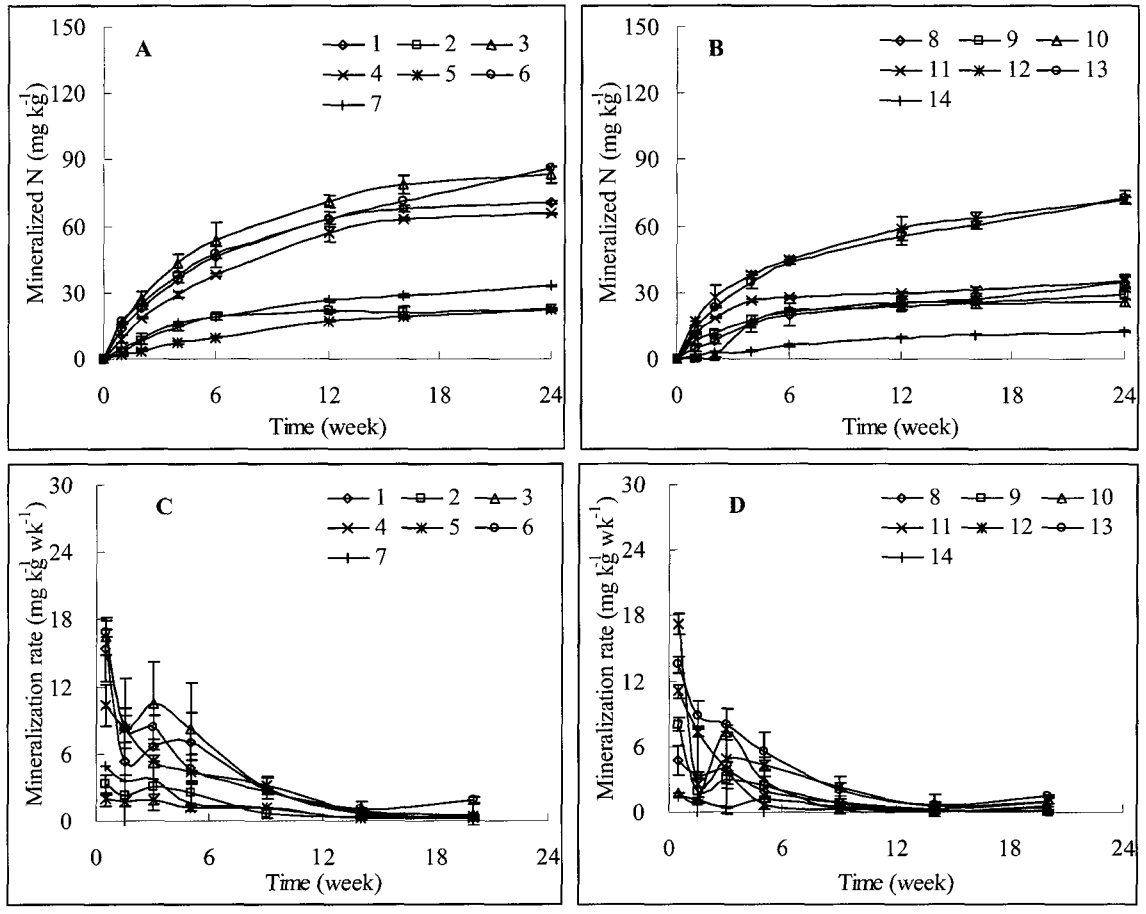

Fig. 2-1. Cumulative mineralizable N released under a 24-week incubation under 15 °C and 55% water holding capacity for soil 1-7 (A) and soil 8-14 (B); and soil N mineralization rate for soil 1-7 (C) and soil 8-14 (D).

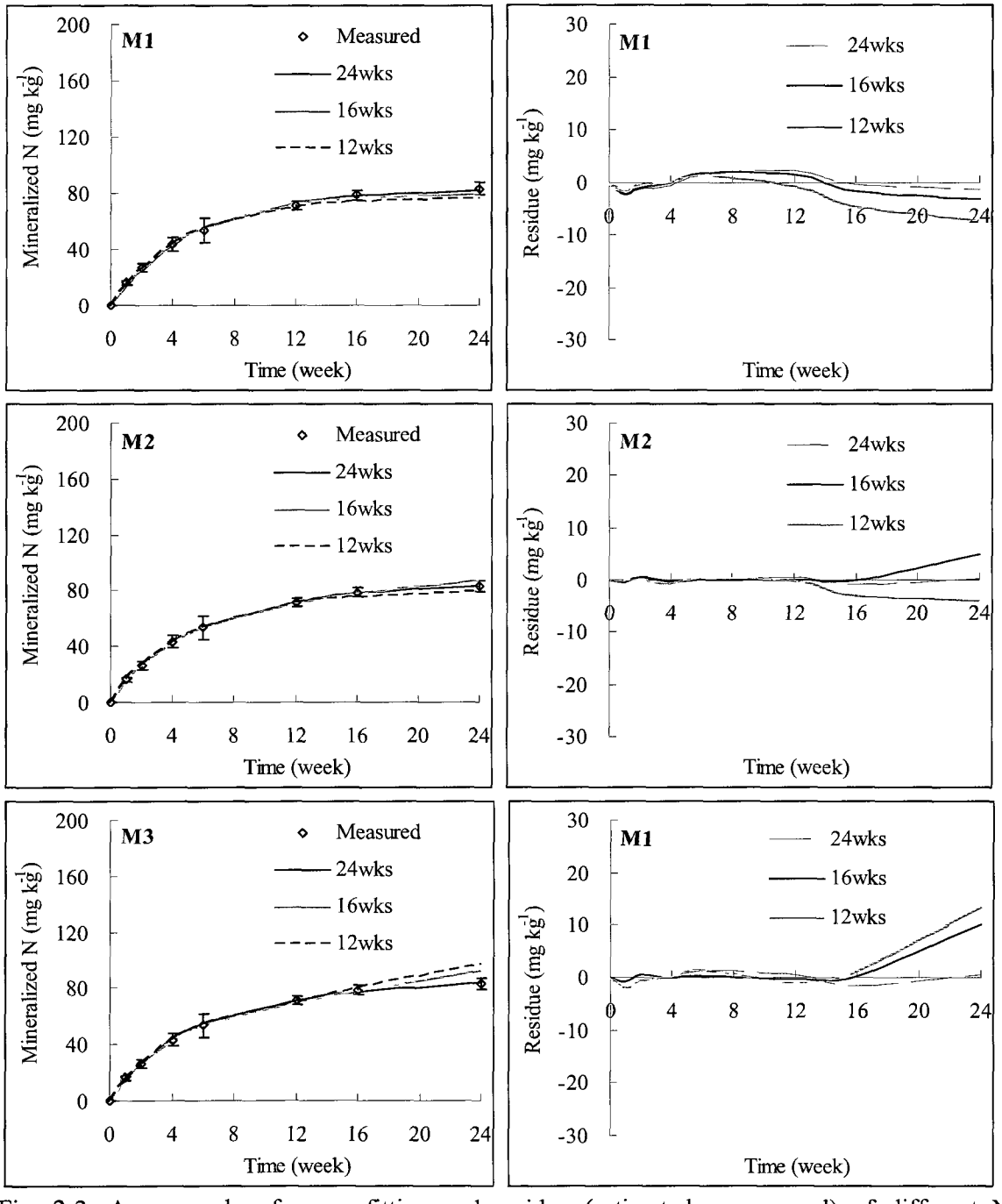

Fig. 2-2. An example of curve fitting and residue (estimated - measured) of different N mineralization models to the N mineralization (soil 3) for different incubation length.

M1, nonfixed simple exponential model: $N_t = N_0 (1\text{-}e^{-kt})$

M2, nonfixed double exponential model: $N_t = N_a (1\text{-}e^{-k_a t}) + N_s (1\text{-}e^{-k_s t})$

M3, nonfixed parallel kinetic model: $N_t = N_a (1\text{-}e^{-kat}) + Kt$

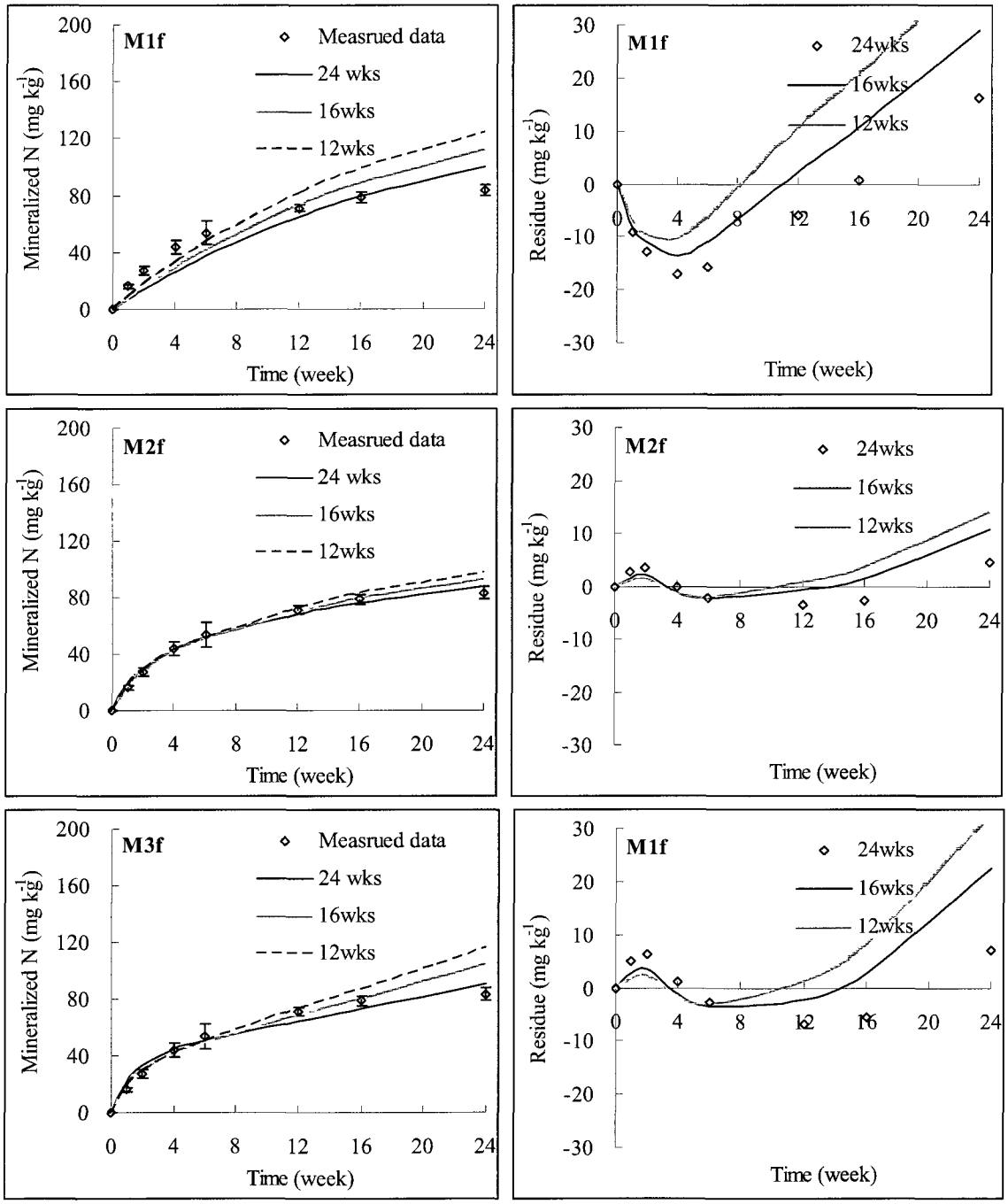

Fig. 2-3 An example of curve fitting and residue (estimated - measured) of different N mineralization models to the N mineralization (soil 3) for different incubation length.
M1f, fixed simple exponential model: $N_t = N_0 (1\text{-}e^{-0.051t})$
M2f, fixed double exponential model: $N_t = N_a (1\text{-}e^{-0.693t}) + N_s (1\text{-}e^{-0.051t})$
M3f, fixed parallel first and zero order kinetic model: $N_t = N_a (1\text{-}e^{-0.693t}) + Kt$

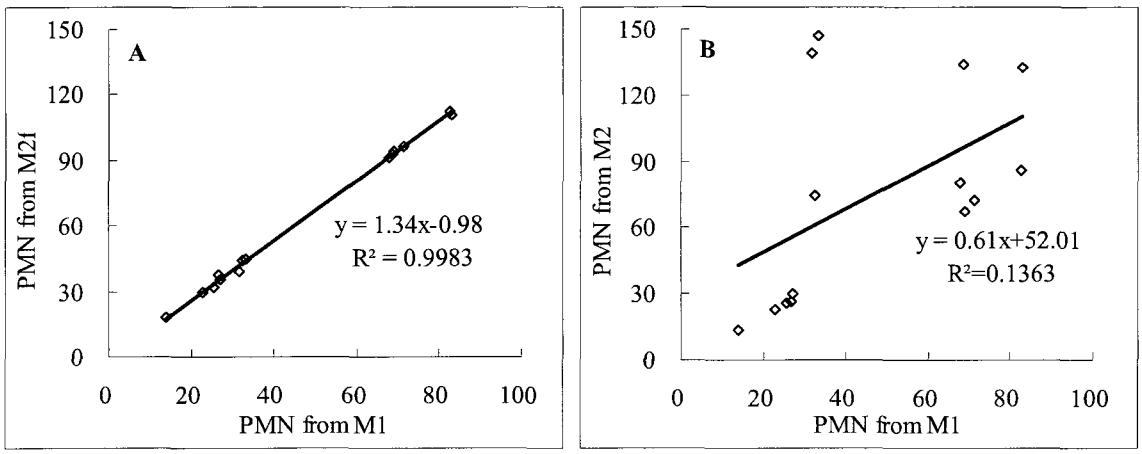

Fig. 2-4 The linear regression between potentially mineralizable N estimated from nonfixed rate constant simple exponential model and that from fixed rate constant double exponential model (A), or that from nonfixed rate constant double exponential model (B).
PMN, potentially mineralizable N. M1, nonfixed rate constant simple exponential model. M2, nonfixed rate constant double exponential model. M2f, fixed rate constant double exponential model with k_a 0.693 wk^{-1} and k_s 0.051 wk^{-1}.

CHAPTER 3 CHEMICAL METHODS AS POTENTIALLY MINERALIZABLE N IN SUBARTIC SOIL[1]

Abstract

A reliable chemical method to predict mineralizable N is useful for making optimal management strategies of N fertilizer and for minimizing adverse impacts of over-use N on the environment. The widely used chemical indices including recently popular methods of sequential cold (22 °C) and hot (80 °C) water extraction were tested for their suitability for routine assessment of mineralizable N in Alaska soils. Fourteen soils were taken from major agricultural areas in subarctic Alaska. The total mineralizable N (N_a + N_s) was estimated using a fixed double exponential model from a 24 week laboratory incubation at 15 °C. Among the all chemical indices, microbial biomass N (MBN), hot water extractable organic N, and NaOH hydrolysable organic N were closely correlated with the total mineralizable N (N_a+N_s), indicating that they were reliable indices for estimating sol mineralizable N.

[1]Zhao, A., Zhang, M., Sparrow, S., Valentine, D. 2011. Chemical methods as potentially mineralizable N in subarctic soil. Prepared for submission in European Journal of Soil Science.

3.1 Introduction

An estimation of N mineralized from soil is important for determining the rate of N fertilizer application to optimize the crop yield and to minimize adverse impacts of N on the environment. In soil, most of N mineralized is from the labile organic N pool (Stanford and Smith, 1972; Parton et al., 1987; Jenkinson et al., 1987). For the past decades, scientists have been developing methods to accurately estimate these labile N pools.

Biological incubation and chemical extractions are two methods used for estimating the size of labile organic N pools. For laboratory incubation, soils usually are incubated for at least 24 weeks under constant soil moisture and temperature. Based on literatures, the optimum soil moisture for net N production normally falls at 55-70% water holding capacity (WHC) (Stanford and Smith, 1972; Linn and Doran, 1984) or between -30 to -10 kPa of water tension (Wang et al., 2003; Stanford and Epstein, 1974; MacDuff and White, 1985). Incubation temperatures vary widely, from 20 to 35 ^{o}C (Christensen and Olesen, 1998; Sharifi et al., 2007; Benedetti and Sebastiani, 1996; Wang et al., 2001; Stanford and Smith, 1972). At higher temperatures, microbial populations can mineralize substrates that are not used at lower temperature (Zogg et al., 1997); thus, incubation at high temperatures may overestimate mineralizable N in soils of cold climates. Hence, to obtain accurate mineralizable N estimators, it is reasonable to use a temperature that is comparable to that in field. In some studies, soils are leached before or during the incubation (Sharifi et al., 2008). However, leaching can remove soluble organic N from soils (Robertson et al., 1988) and overestimate potentially mineralizable N pool sizes

because the leachate N may include mineral N that could have been immobilized (Wang et al., 2003). A non-leaching approach might be more appropriate for the prediction of soil N production under field conditions.

Many models have been used to fit data from laboratory incubations to estimate soil mineralizable N. The popular models include single exponential models, parallel models, and double exponential models (Stanford and Smith, 1972; Molina et al., 1980; Bonde and Rosswall, 1987). Recently, single and double exponential models have been modified with fixed rate constants to standardize the estimation of soil mineralizable N (Wang et al., 2003; Wang et al., 2004).

Laboratory incubation has been considered the best predictor of mineralizable N but it is laborious and time consuming. Chemical methods can distinguish soil organic matter fractions based on their respective solubility in various extractants or spectroscopic characterization in extracted solution (Coŭteaux et al., 2003; Kalbitz et al., 2003). The simple and fast chemical extractions have been sought that would accurately predict mineralizable N in soils. In general, a chemical method is considered to be best for estimating mineralizable N if there is a high correlation between this chemically extractable N and soil mineralizable N estimated by a kinetic model that can best fit to data from laboratory incubation. The commonly used chemical extractants include acid (e.g. 1 *M* HCl), alkaline (e.g. 1 *M* NaOH), neutral (e.g. 2 *M* KCl), cold DI water at room temperature, or hot DI water at 80 °C (Wang et al., 2001; Curtin and Wen, 1999; Curtin et al., 2006). Spectroscopy analysis following chemical extractions also is used to correlate with potentially mineralizable N such as ultraviolet absorbance at wavelength

205 nm and 260 nm of 0.01 *M* $NaHCO_3$ extraction (Fox and Piekielek, 1978). In theory, the mild extractants (water) are superior to harsh chemicals (acid, alkaline) because mild extractant extracts a smaller amount of non-labile N than the harsh chemicals (Curtin et al., 2006). The water extractable organic N is reported to be a good indicator of soil N availability compared with total N and mineralizable N during 7 d anaerobic incubation and a 28 d aerobic incubation (Curtin et al., 2006). However, little information is available on whether water extractable organic N as N availability is any better than other widely used chemical indices.

The objectives of this study were: 1) to determine the relationships between water extractable organic N (22 and 80 °C) and the other chemically determined organic N; and, 2) to find the best chemical indices that is suitable to predict mineralizable N for Alaska soils.

3.2 Materials and methods

3.2.1 Soils

We took fourteen soil samples at major agricultural areas (Fairbanks, Delta Junction, and Palmer) of subarctic Alaska, USA., representing different land uses (Conservation Reserve Program (CRP), forest, agriculture). The Conservation Reserve Program is administrated by USDA and aimed to protect environmently sensitive agricultural land (eg. highly erodible land) by converting these agricultural lands into vegetative cover lands. The soil series in my study included Tanana (coarse-loamy, mixed, superactive, subgelic Typic Aquiturbels), Nenana (coarse-silty over sandy or sandy-skeletal, mixed, superactive Typic Haplocryepts), Volkmar (coarse-silty over

sandy or sandy-skeletal, mixed superactive Aquic Haplocryepts), and Knik (coarse-silty over sandy or sandy-skeletal, mixed, superactive Typic Haplocryepts). Nine of the fourteen soil samples were taken from Delta Junction area. The soil samples (0-10 cm) were taken from three sites in September of 2005. Each site has three land uses: forest, CRP, and agriculture. No lime was applied in all land uses. A portion of fresh soil samples were used for measurement of microbial biomass C and N. The other portion of fresh soils was air dried. The remaining five out of fourteen samples were from the samples taken for other purposes (2007). Samples were individually taken at 0-15 cm soil surface from Fairbanks Experiment Farm, Matanuska Experiment Farm at Palmer, Delta Junction Field Research Site, and Rosie Creek Farm near Fairbanks. All soil samples were air dried and sieved (<2 mm) before analysis. Soil pH was determined in a 1:1 soil/water suspension using a soil pH meter 140 (Corning Inc., New York, USA). Soil texture was analyzed by the hydrometer method after dispersion with sodium hexametaphosphate. Soil total C and N was measured by thermal combustion analysis using a LECO Truspec CN analyzer (LECO Corporation., St. Joseph MI, USA). The results were shown in Table 3-1.

3.2.2 Laboratory incubation to determine soil mineralizable organic N

Each soil sample was done in triplicate for laboratory incubation (a total sample number of 14 $\times$ 3). The incubation followed the procedure used by Wang et al. (2004). Briefly, each soil sample (5.00 g) was weighed into a 50 mL glass vial and moistured with distilled water (55% water holding capacity). The vials were incubated for 24 weeks at 15 ^{o}C. During the incubation, soil moisture content was monitored weekly and the caps

of glass vials were loosely capped in order to allow aeration. Soil samples were destructively collected at 0, 1, 2, 4, 6, 9, 12, 16, and 24 weeks. At each time, soil (1.00 g) was extracted by 10 ml 2 *M* KCl and inorganic N (NH_4^+-N + NO_3^--N) in the extracted solution was determined using continuous flow analysis technique by an Alpkem Rapid Flow Analyzer (Astoria-Pacific International, Oregon, USA).

3.2.3 Chemical methods to determined mineralizable N

All chemical extractions were done in triplicate. The extractable N was calculated on oven dried basis (24 h, 105 °C), unless otherwise stated.

3.2.3.1 2 *M* KCl extractable N

One gram of collected air-dry samples was weighed into centrifuge tubes and suspended in 10 mL 2 *M* KCl. The tubes were shaken for 30 min on an Eberbach 6000 reciprocal shaker (Eberbach Corporation, Michigan, USA) at room temperature (22 ±1 °C). Then, the extracted solutions were obtained through filtration of about 2.5 μm filters (Whatman No.42 Ashless Filter, Whatman International Ltd, England). Inorganic N concentrations (NH_4^+-N + NO_3^--N) in extracted solutions were determined by continuous flow analysis technique using an Alpkem Rapid Flow Analyzer.

3.2.3.2 Sequential extraction of water extractable organic N (22 °C, 80 °C)

Cold and hot water extractable organic N were sequentially extracted following the procedure used by Curtin et al. (2006). Briefly, four grams of soil samples with 30 mL of deionized water were shaken in 50 mL centrifuge tubes for 30 min at 22 °C. The tubes were then centrifuged at 900×g, followed by filtration through about 2.5 μm filter papers. The centrifuge tube plus wet soils was weighed to calculate the entrained water volume.

Another 30 mL deionized water was added into tubes and the tubes were placed in a hot water bath at 80 °C for 16 h. The tubes were then centrifuged and filtered as the extraction of cold water extractable N. Total extractable C and N in cold and hot water were determined using a Shimadzu TOC-V Organic Carbon and Total Nitrogen Analyzer (Shimadzu Scientific Instruments Inc., Columbia, USA). Extractable inorganic N in cold and hot water was measured using an Alpkem Rapid Flow Analyzer. The extractable organic N in cold and hot water was calculated by the difference of total extractable N and inorganic N.

3.2.3.3 0.01 *M* $NaHCO_3$ extractable N

The extraction of 0.01 *M* $NaHCO_3$ followed the procedure of Fox and Pierkielek (1978) and Hong et al. (1990). Briefly, 2.5 g of soil was shaken in 50 mL of 0.01 *M* $NaHCO_3$ for 15 min in a 50 mL centrifuge tube. The suspension was filtered through a 2.5 μm filter paper and ultraviolet (UV) absorbance of the extract was measured at 205 ($NaHCO_3$_205) and 260 nm ($NaHCO_3$_260) with a UV-VIS-NIR spectrophotometer UV-3600 (Shimadzu Scientific Instruments Inc., Columbia, USA).

3.2.3.4 1 *M* HCl extractable N

The extraction using 1 *M* HCl followed the procedure modified by Xu et al. (1997). Ten grams of soil was weighed into a 70 mL digestion tube and mixed with 50 mL 1 *M* HCl. The tube was covered with a small glass cap and heated on a block digester at 100 °C. After 4 h, the tube was allowed to cool to room temperature and the suspension was filtered through 2.5 μm filter paper. A 20 mL aliquot of the filtrate was pipetted into a 50 mL beaker, neutralized to pH 6.5 with 2 *M* NaOH, and transferred to a 100 mL

volumetric flask and diluted with deionized water. During the extraction, the 1 *M* HCl transformed the hydrolyzable organic N mostly into NH_4^+-N. The NH_4^+-N was then analyzed with the Alpkem Rapid Flow Analyzer. The HCl-hydrolysable N was obtained by subtracting the initial NH_4^+-N from the total amount of NH_4^+-N after hydrolysis.

3.2.3.5 1 *M* NaOH extractable N

The analysis of 1 *M* NaOH hydrolysable organic N was carried out following the procedure described by Wang and Li (1991). A soil sample (4.00 g) was weighed into a 200 mL wide mouth glass jar. Then, 10 mL 1 *M* NaOH was added to the jar with soil and a glass beaker with 10 mL H_3BO_3 indicator solution was placed into the jar, which was sealed immediately. The jar was incubated at 40 °C in an incubator for 24 h. The amount of NH_4^+-N released was determined by titration of the indicator with standard 0.0025 *M* H_2SO_4. The NaOH hydrolysable N was calculated by the difference of NH_4^+-N before and after NaOH treatment.

3.2.3.6 Microbial biomass C and N

Nine soil samples were used for determination of soil microbial biomass C (MBC) and N (MBN) since the others were archived air dry samples. The chloroform fumigation extractable C and N were determined as an index of soil MBC and MBN. Field moisture soil samples taken from Delta Junction were sieved through a 2 mm screen. About 10.00 g fresh soil was weighed out into two separate extraction glass bottles.

The soil (10.00 g) in one glass bottle was added to 50 ml 0.5 *M* K_2SO_4 and shaken for 30 min, followed by filtering through VWR410 filter paper, which had been rinsed

twice with DI water and once with 0.5 *M* K_2SO_4 into 50 ml centrifuge tubes. The soil in the other glass bottle was fumigated with ethanol-free chloroform for 24 h at room temperature in the dark under vacuum (711 mm Hg or 14 psi.) in a desiccator lined with moist paper towels. After purging the chloroform by three repeated evacuations of the desiccator, fumigated soils were extracted with the same procedure used for nonfumigated soils. The samples were refrigerated at 4 °C until analysis. The fumigated and nonfumigated extractions were used for determination of microbial biomass C following the potassium dichromate method (Vance et al., 1987; Ross, 1989) and microbial biomass N following the persulfate method (Brookes et al., 1985). The amount of C and N extracted in the unfumigated soil was substracted from the amount in the fumigated soil and the result divided by conversion factor 0.38 (fraction of biomass C mineralized to CO_2) for biomass C and by conversion factor of 0.54 (converting the chloroform-labile N pool into the microbial biomass N) for biomass N (Vance et al., 1987).

3.2.4 Models to determine soil mineralizable organic N

To determine the potentially mineralizable N, cumulative inorganic N in laboratory incubations was fitted by different kinetic models. Among these models, double exponential model with fixed k_a 0.693 $weeks^{-1}$ and k_s 0.051 $weeks^{-1}$ was best to estimate soil mineralizable N (see Chapter 2). Therefore, we used a fixed double exponential model to estimate soil mineralizable N.

M2f: Double exponential model with fixed k_a value of 0.693 $weeks^{-1}$ and k_s value of 0.051 $weeks^{-1}$.

$$N_t = N_a(1-e^{-0.693t}) + N_s(1-e^{-0.051t}) \qquad (3.1),$$

where two pools are used to describe the readily mineralizable N_a (mg kg^{-1}) and the less readily mineralizable N_s (mg kg^{-1}).

3.2.5 Statistical analyses

All correlation and regression analyses were done with Matlab (Version 7.1, The MathWorks Inc., Massachusetts, USA). Soil mineralizable N was estimated with nonlinear regression using the Marquardt algorithm. Pearson correlation was used to relate the N extracted by the various chemical methods. Linear regression analyses were used to determine the association among model determined mineralizable N and chemically extracted N. The linear regression was conducted to relate mineralizable N pool (N_a, N_s, N_a+N_s) with other chemical indices (NaOH hydrolysable N, HCl hydrolysable N, $NaHCO_3$_205, $NaHCO_3$_260, hot water extractable organic C, N, cold water extractable organic C, N, total C, and total organic N).

3.3 Results and Discussion

The soils used in this study represented a wide range of pH (5.0-6.9), total organic C (22-83 g kg^{-1}), total organic N (1.7-5.0 g kg^{-1}), and C:N ratio (12-24).

3.3.1 Incubation and model determined mineralizable N

During a 24-week incubation, total mineralizable N in the 14 soils ($N_{24weeks}$) ranged from 12 to 86 mg kg^{-1} with a CV of 56% (Table 3-2). The $N_{24weeks}$ accounted for between 0.6% and 4.4% (average of 2%) of total organic N.

The active mineralizable N pool (N_a) size estimated from fixed double exponential model ranged from 1.9 to 30.2 mg N kg^{-1} with an average of 16.5 mg N kg^{-1}. The slow

mineralizable N pool (N_s) ranged from 18.9 to 120.5 mg N kg^{-1} with an average of 57.2 mg N kg^{-1}. The N_a averaged only 26% of the total (N_a+N_s). The N_{24wk} can explain an average of 64% of total mineralizable N (N_a+N_s). The total of N_a and N_s averaged2.8% of the total organic N.

The percentage of total mineralizable N pool sizes (N_a+N_s) in total organic N (3.2%) was lower than reported by Sharifi et al. (2007; 6%), Sharifi et al. (2008, 7.5%), and Benedetti and Sebastiani (1996, 10%). This may be caused by at least three reasons. First, the incubation in this study was at 15 °C, which was lower than other studies (25 °C for Sharifi et al., 2007; 30 °C for Benedetti and Sebastiani, 1996; 35 °C for Schomberg et al., 2009). The rate of mineralization increases exponentially with increase in temperature within 5 to 35 °C (Stanford et al., 1972). Second, leaching procedure before incubation can lead to overestimation of potentially mineralizable N pool sizes because the leachate N may include mineral N that could have been immobilized (Wang et al., 2003). This study did not use leaching procedure and hence had lower N_0 than previous studies (Benedetti and Sebastiani, 1996; Sharifi et al., 2007). Third, C:N ratios of soils here (12-24) were higher than that reported by Benedetti and Sebastiani (1996, 7-15) and Sharifi et al. (2007, 7-13), which may result in less net N mineralization in soils of this study.

3.3.2 Chemically determined mineralizable N

The organic N and C content in hot water ranged from 36 to 104 (mean = 63) mg N kg^{-1} and from 609 to 1701 (mean=1121) mg C kg^{-1} (Table 3-2). The hot water extractable organic N and C accounted for 2.9% (1.4%-4.7%) and 2.7% (1.5%-4.4%) of total organic N and C. The C:N ratio (12.6-23.4) of hot water extracted solutions paralleled that of the

whole soils (12.3-24.0). Chantigny et al. (2010) also found a similarity in C:N ratios of hot water extractable organic matter (8.2-22.7) and whole soils (11.0-22.1); in these soils across forest, grassland and arable lands, hot water extracted 2.2-5.4% of total N and 1.6-4.7% of total C. However, some studies from pasture and arable soils have reported that hot water extracts narrower C:N ratios than whole soil organic matter (Gregorich et al., 2003; Curtin et al., 2006).

The organic N and C content in cold water ranged from 10 to 47 (mean of 24 mg N kg^{-1}) and from 274 to 756 (mean of 438 mg C kg^{-1}) (Table 3-2). The cold water extractable organic N and C accounted for 1.1% (0.5%-1.6%) and 1.1% (0.5%-2.0%) of total organic N. The C:N ratios of cold water extractable organic matter averaged 18.7 ranging from 13.4 to 26.5. The percentage of cold water extactable N in total organic N was within the range of 0.5-2.5% in arable soils (Wang et al., 2001) and 0.2-1.4% in soils across forest, grassland and arable lands (Chantigny et al., 2010). The values of cold water extractable C were also similar to reported by Curtin et al. (2006; 164 -634 mg C kg^{-1} in pasture and arable soils), and Gregorich et al. (2003; 280 to 570 mg C kg^{-1} in maize-cropped soils). The hot water extractable C and N were 2.6 times higher than cold water extractable C and N.

The NaOH hydrolysable N content ranged from 35 to 258 (mean = 133) mg N kg^{-1} (Table 3-2). The NaOH hydrolysable N accounted for 5.9% (3.6%-7.7%) of total organic N. The procedure to extract NaOH hydrolysable N is also used by Wang et al. (2001) in 19 arable soils. The values of NaOH hydrolysable N here were similar with those reported of 30 to 400 mg N kg^{-1} by Wang et al. (2001). The NaOH hydrolysable N

extraction is similar to the procedure used for Illinois soil nitrogen test (ISNT), except for an extraction of extractable NH_4^+-N (Sharifi et al., 2007). The ISNT can recover amino sugar N derived primarily from bacterial and fungal cell walls and some α-amino acid (Khan et al., 2001, Greenfield, 2001). The NaOH hydrolysable N extracted the highest proportion of total organic N in soils among the chemical methods used.

The HCl hydrolysable N content ranged from 36 to 156 (mean = 73) mg N kg^{-1} (Table 3-2). The HCl hydrolysable N accounted for 3.3% (1.4%-5.3%) of total organic N. These values were similar with reported of 25 to 150 mg N kg^{-1} in 19 arable soils by Wang et al. (2001).

The UV absorbance that can be used to estimate dissolved organic matter is also used to predict the potentially mineralizable N in soils. The UV value at 260 nm is positively related with the amount of dissolved organic matter contained in the soil, and the UV at around 200 or 205 is positively related with the amount of dissolved organic matter plus NO_3^- content in the soil (Hong et al., 1990; Serna and Pomares, 1992). The UV absorbance of 0.01 *M* $NaHCO_3$ ranged from 0.46 to 0.96 with an average 0.65 mg N kg^{-1} at 260 nm, and from 0.93 to 2.66 with an average 1.55 mg N kg^{-1} at 205 nm (Table 3-2).

Nine soil samples were used for determination of soil MBC and MBN because the others were archived air dry samples. The MBC and MBN averaged 49 mg C kg^{-1} (21 to 78 mg C kg^{-1}), and averaged 18 mg N kg^{-1} (4 to 29 mg N kg^{-1}) (Table 3-2). The MBC and MBN accounted for 0.1% (0.05%-0.14%) and 0.39% (0.1%-0.6%) of total organic N, respectively.

3.3.3 Correlations between water extractable C and N with the other chemically determined mineralizable N

Cold and hot water extractable organic C and N were highly correlated (r=0.89, p<0.01) (Table 3-3). The cold water extractable organic N was highly correlated with HCl hydrolysable N (r=0.80, p<0.01) , UV absorbance at 260 nm of $NaHCO_3$ extracted solutions (r=0.83, p<0.01), and microbial biomass N (r=0.83, p<0.01). The hot water extractable organic N was highly correlated with HCl hydrolysable N (r=0.86, p<0.01) and microbial biomass N (r=0.89, p<0.01). The hot water extractable organic N was also significantly (r=0.61, p<0.05) correlated with UV absorbance at 260 nm of $NaHCO_3$ extracted solutions. Both cold and hot water extractable organic C and N (22 and 80 °C) were poorly correlated with NaOH hydrolysable organic N, UV absorbance at 205 nm of $NaHCO_3$ extracted solutions, MBC, total C and total organic N.

Earlier studies reported poor correlations between water extractable organic C and N (22°C) (Campbell et al., 2000; Curtin et al., 2006). The poor correlation between water extractable organic C and N is assumed to result from the difference in factors driving dissolved organic C and N production such as N fertilization and ecosystem age (McDowell, 2003). For example, N fertilization may increase dissolved organic N but not dissolved organic C. In this study, samples were taken before N fertilization. Apparently, a good correlation of water extractable organic C and N (22 °C) reflected little influence of factors such as N fertilization or soil age on dissolved organic C and N production. A high correlation between water extractable organic C and N (80 °C) has

been reported by Curtin et al. (2006) and Wang et al. (2007). A high correlation between water extractable organic C and N (80 °C) was also found in this study.

Some of the compounds of dissolved organic N or cold water extractable organic N comprise transformed plant derived polysaccharides and microbial metabolites (Qualls and Haines, 1991). Especially, the dissolved organic matter of air dried soils might contain a substantial portion of killed soil microorganism biomass during the drying process (Wang et al., 2001). Here, the cold water extractable organic N was determined in air dried soils. A high correlation between water extractable organic N (22 °C) and MBN may reflect a large contribution of microbial N to cold water extractable organic N. Sparling et al. (1998) suggested that hot water extractable organic matter is predominantly of microbial origin. A close correlation between hot water organic N and MBN is also reported by Wang and Wang (2007) for soils planted with native broadleaf forest and pure coniferous plantation in Southern China. Similarly, we also found a high correlation between hot water extractable organic N and MBN.

Dissolved organic N or cold water extractable organic N often consists of labile hydrophilic compounds (e.g. free amino acids, amino sugars, small carbonxylic acids, proteins, sugars) due to the selective sorption of recalcitrant aromatic and hydrophobic compounds by the solid fraction of organic matter from the soil solution (Qualls and Haines, 1991; Guggenberger and Kaiser, 2003; Kalbitz et al., 2003). The hot-water extractable organic matter contains dead microbe, soluble soil carbohydrates and other simple compounds which may account for the labile fraction of SOM (Ghani et al., 2003). The HCl hydrolysable organic N and NaOH hydrolysable organic N are assumed to be

indicators of biologically active soil organic matter (Xu et al., 1997; Wang et al., 2001). Total organic C and N in soil organic matter include all labile and recalcitrant organic C and N and the recalcitrant organic C and N usually account for most of total C and N. As such, total C and N here showed no significant ($p>0.05$) correlation with the water extractable organic C or N representing labile organic C and N fractions. Water extractable organic N (22, 80 °C), HCl hydrolysable organic N and NaOH hydrolysable organic N were similar in their extraction of labile organic N, which might account for a significant ($p<0.05$) correlation between water extractable organic N and HCl or NaOH hydrolysable organic N.

The UV_{260} of $NaHCO_3$ indicates the amount of water extractable organic matter but the UV_{205} of $NaHCO_3$ reflects the amount of inorganic and organic matter in water extraction (Hong et al., 1990; Serna and Pomares, 1992). Hence, the correlation of water extractable organic matter (22 °C) was higher with UV_{260} of $NaHCO_3$ than UV_{205} of $NaHCO_3$ in this study. In general, the UV absorbance at long wavelength determines the aromaticity of organic matter. Compared with water extractable organic matter (22 °C), water extractable organic matter (80 °C) showed a poor correlation with UV values at 260 and 205 nm of $NaHCO_3$, which may reflect a low aromaticity of hot water extractable organic matter.

3.3.4 Relationships between chemically extractable organic N with model determined mineralizable N

The N_a was evaluated for its relationship with chemically determined organic N (Table 3-4). N_a was significantly ($p<0.05$) correlated with MBN, hot water extractable

organic N, NaOH hydrolysable organic N, and cold water extractable organic N. However, N_a was only highly correlated with MBN (R^2=0.74, p=0.003).

The N_a+N_s was significantly (p<0.05) related to MBN, hot water organic C and N, cold water organic C and N, NaOH hydrolysable N, and HCl hydrolysable N (Table 3-4). However, only MBN, hot water organic N, and NaOH hydrolysable N were highly correlated (R^2=0.80, 0.70, 0.62) with the N_a+N_s (Fig. 3-1). There was no significant (p>0.05) relationship between N_a+N_s and other chemically determined organic N including UV absorbance at 205 and 260 nm of $NaHCO_3$ extracted solution, total organic C and N (Table 3-4).

The measurement of microbial biomass usually requires fresh soil samples, which often need to be analyzed immediately or refrigerated at low temperature for a short time before analysis. In contrast, hot water organic N and NaOH hydrolysable N use air dry soils which can usually be stored for a longer time with more stable than fresh soil. To obtain a reliable relationship between chemically determined organic N and potentially mineralizable organic N from incubation, a large number of soil samples are required. These samples probably can not be measured in time due to shortage of labor or others, leading to a great change in MBN. Therefore, taking into account of costs of labor and time, we think that hot water organic N and NaOH hydrolysable N may be a better choice to use as indicators of potentially mineralizable N than MBN. The correlation of NaOH hydrolysable N with N_0 was lower than that of hot water organic N with N_0. Therefore, hot water organic N was a very promising method for routine prediction of N mineralization potential in subarctic soils. Curtin et al. (2006) found that hot water

extractable organic N was reported as a reliable predictor of potentially available N measured by greenhouse-grown oat (*Avena sativa* L.) crop N uptake.

3.4 Conclusions

The cold and hot water extractable organic C and N were highly correlated in subarctic soils. The cold and hot water extractable organic N was highly correlated with microbial biomass N, NaOH hydrolysable N, HCl extractable hydrolysable N. The cold water extractable organic N was also highly correlated with UV absorbance at 260 nm of $NaHCO_3$ extracted solutions.

Based on correlation of N_a+N_s with chemically determined organic N, we found that the MBN, hot water extractable organic N, and NaOH hydrolysable organic N were the best to predict potentially mineralizable organic N. However, the measurement of MBN is based on fresh soils, leading to a problem of sample handling and storage. In the respect of easily handling and storage, hot water organic N based on air dry soils may be superior for a routine soil N supply capacity test in subarctic soils. Further studies are needed to test the ability of hot water extractable organic N to estimate N mineralization capacity of field soils.

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Table 3-1. Summary of selected chemical and physical characteristics of 14 soils used in this study

Soil	Land use	Location	Texture	pH[1]	Total C g kg^{-1}	Total N g kg^{-1}	Soil C:N
1	CRP[2]	Delta Junction	Sandy loam	5.4	45.0	2.00	22.5
2	Forest	Delta Junction	Sandy loam	5.2	47.0	2.00	23.6
3	Agriculture	Delta Junction	Sandy loam	5.4	57.0	2.97	19.2
4	CRP	Delta Junction	Sandy loam	5.4	41.0	1.99	20.6
5	Forest	Delta Junction	Loam	5.4	48.0	2.00	24.0
6	Agriculture	Delta Junction	Loam	5.4	38.0	1.98	19.2
7	Agriculture	Palmer	Loam	5.3	31.4	2.00	15.7
8	Agriculture	Delta Junction	Loam	5.1	33.0	1.69	19.6
9	Agriculture	Fairbanks	Loam	6.9	22.6	1.83	12.3
10	Agriculture	Fairbanks	Loam	6.2	29.0	1.86	15.5
11	Agriculture	Fairbanks	Sandy loam	6.7	36.5	2.00	18.2
12	CRP	Delta Junction	Loam	5.3	83.0	4.99	16.6
13	Agriculture	Delta Junction	Loam	5.0	50.0	2.98	16.8
14	Forest	Delta Junction	Loam	5.4	48.0	2.00	24.0

[1] DI water (solution:soil=1:1 (v:w)).
2 Conservation Reserve Program.

Table 3-2. Mean, standard deviation and range of values for indices of N mineralization in Alaska soils

Index	Mean	SD[1]	Minimum	Maximum	C.V.%[2]
$N_{24weeks}$[3], mg N kg^{-1}	47.50	26	12.33	86.09	56
$N_{24weeks}$/total organic N, %	2.07	1.06	0.62	4.30	51
N_a[4], mg N kg^{-1}	16.5	9.0	1.9	30.2	55
N_s[5], mg N kg^{-1}	57.2	30.8	18.9	120.5	54
(N_a+N_s)/total organic N, %	2.75	1.43	0.89	5.56	52
Organic N_hot water, mg N kg^{-1}	63	20	36	104	31
Organic C_hot water, mg N kg^{-1}	1121	383	609	1701	34
Organic N_cold water,mg N kg^{-1}	24	9	10	47	35
Organic C_cold water, mg N kg^{-1}	438	125	274	756	28
NaOH hydrolyzable N, mg N kg^{-1}	133	49	91	258	37
HCl hydrolyzable N, mg N kg^{-1}	73	31	36	156	43
$NaHCO_3$_260, UV absorbance	0.65	0.12	0.46	0.96	18
$NaHCO_3$_205, UV absorbance	1.55	0.55	0.93	2.66	36
Microbial biomass C, mg C kg^{-1}	49	17	21	78	34
Microbial biomass N, mg N kg^{-1}	10	4	2	15	42

[1] Standard deviation.
[2] Coefficient of variance.
[3] The mineralized N during a 24 weeks incubation.
[4] The labile N_a pool estimated from modified exponential model.
[5] The labile N_s pool estimated from modified exponential model.

Table 3-3. Pearson correlation coefficient between water extractable organic C and N and the other indices of N mineralization

Index	[1]HWOEN	[2]HWOEC	[3]CWOEN	[4]CWOEC
[1]HWOEN, mg N kg^{-1}	1			
[2]HWOEC, mg N kg^{-1}	0.89 **	1		
[3]CWOEN, mg N kg^{-1}	0.80 **	0.53 *	1	
[4]CWEOC， mg N kg^{-1}	0.84 **	0.72 **	0.89 **	1
NaOH hydrolysable N, mg N kg^{-1}	0.72 **	0.66 **	0.63*	0.50
HCl hydrolysable N, mg N kg^{-1}	0.86 **	0.84 **	0.80 **	0.93 **
$NaHCO_3$_260, UV absorbance	0.61 *	0.47	0.83 **	0.81 **
$NaHCO_3$_205, UV absorbance	0.27	0.01	0.59 *	0.41
[5]MBC, mg N kg^{-1}	0.88 **	0.68 *	0.83 **	0.66 *
[6]TC, g kg^{-1}	0.15	0.50	0.21	0.37
[7]TN, g kg^{-1}	0.09	0.22	0.29	0.26
[8]MBC, mg C kg^{-1}	0.23	0.15	-0.09	0.10

* Significant correlation at $p<0.05$, ** Significant correlation at $p<0.001$.
[1]Organic N in hot water
[2]Organic C in hot water
[3]Organic N in cold water
[4]Organic C in cold water
[5]Microbial biomass N
[6]Total C
[7]Total organic N
[8]Microbial biomass C

Table 3-4. Linear regression of tested indices of soil N mineralization to model estimated mineralizable N.

Index	N_a[1] mg N kg^{-1} soil		N_a+N_s[2] mg N kg^{-1} soil	
	R^2	*p* value	R^2	*p* value
MBN[3], mg N kg^{-1} soil	0.74	0.003	0.80	0.0009
Organic N_hot water, mg N kg^{-1} soil	0.42	0.01	0.70	0.0002
NaOH hydrolyzable N, mg N kg^{-1} soil	0.40	0.01	0.62	0.0008
Organic C_hot water, mg N kg^{-1} soil	0.17	0.14	0.46	0.007
Organic N_cold water, mg N kg^{-1} soil	0.36	0.02	0.43	0.01
Organic C_cold water, mg N kg^{-1} soil	0.14	0.18	0.32	0.03
HCl hydrolyzable N, mg N kg^{-1} soil	0.14	0.18	0.32	0.04
$NaHCO_3$_260, UV absorbance	0.14	0.18	0.13	0.19
$NaHCO_3$_205, UV absorbance	0.28	0.05	0.12	0.22
Total C, g kg^{-1}	0.2	0.11	0.18	0.79
Total organic N, g kg^{-1}	0.33	0.03	0.21	0.73
MBC[4], mg N kg^{-1} soil	0.26	0.16	0.22	0.22

[1] The labile organic N pool size estimated from fixed double exponential model.
[2] The total of N_a and N_s estimated from fixed double exponential model.
[3] Microbial biomass N.
[4] Microbial biomass C.

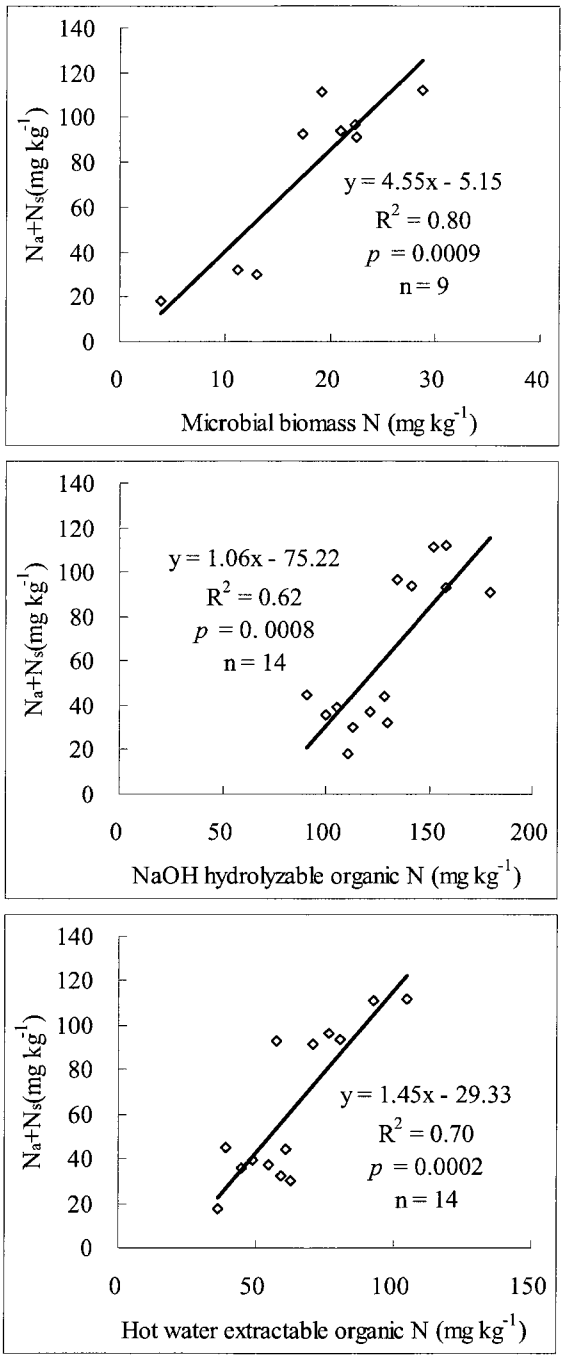

Fig. 3-1. Linear relationships between potentially mineralizable N (N_a+N_s) and microbial biomass N, hot water extractable organic N, and NaOH hydrolysable organic N.

CHAPTER 4 SPECTROSCOPIC CHARACTERISTICS AND BIODEGRADABILITY OF COLD AND HOT WATER EXTRACTABLE ORGANIC MATTER[1]

Abstract

Cold (22°C) and hot water (80 °C) extractions have been used to estimate the available organic C and N in soils. However, it is not clear which extractant is more labile than the other. The aim of this study was to assess the concentration, chemical characteristics, and biodegradability of cold and hot water extractions. Chemical characteristics were determined by UV absorptivity at 254 nm (SUV_{254}), humification index (HIX), fluorescence indx (FIX), fluorescence efficiency (F_{eff}), and fluorescence components. Degradability was assessed in a 21-d solution incubation by measuring the loss of water extractable organic C (WEOC) and N (WEON). In all soils, the concentrations of WEOC and WEON were significantly ($p<0.05$) higher in hot than cold water extractions. The cold and hot WEOM consisted of humic-like, fulvic-like, and protein-like fluorophores. However, the types of protein-like components in cold and hot WEOM were different with trypotophan-like component in cold WEOM and tyrosine-like component in hot WEOM. The SUV_{254} and HIX were significantly ($p<0.05$) decreased but the F_{eff}, humific-like, and protein-like components were significantly ($p<0.05$) increased in hot water extractions of all soils. The biodegradability of hot WEOC and WEON was significantly ($p<0.05$) higher than that of cold WEOC and WEON in all soils. The biodegradability of cold or hot WEOC was significantly

correlated ($r=0.78$, $p<0.01$) with the protein-like component, indicating that a protein-like fluorophore is a labile fraction in cold and hot WEOM pools.

[1] Zhao, A., Zhang, M., Valentine, D. 2011. Spectroscopic characteristics and biodegradability of cold and water extractable organic C and N. Prepared for submission in Biology and Fertility of Soils.

4.1 Introduction

Water extractable organic matter (WEOM) or dissolved organic matter (DOM) is a labile substrate for microbes in soil (Burford and Bremmer, 1975). The release and turnover of WEOM play an important role in soil carbon (C) and nitrogen (N) cycling (Schimel and Bennett, 2004; Kalbitz, 2000). Some researchers extract WEOM at room temperature (cold WEOM) (Corre et al., 1999; Davidson et al., 1987; van Ginkel et al., 1994; Jandl and Sollins, 1997). Others obtain WEOM at 80 °C (hot WEOM) (Sparling et al., 1998; Gregorich et al., 2003). Extraction temperature affects solubility of soil organic matter in water, and relase of protected soil microbial biomass into solution (Ros et al., 2009). Knowledge of impact of extraction temperature on WEOM is crucial to understand the role of WEOM in soil C and N cycling and describe the dynamics of C and N in soils (Chantigny et al., 2010).

The influence of extraction temperature on quantity of WEOM has been widely investigated in soils. Chantigny et al. (2010) reported that water extractable organic C (WEOC) and N (WEON) increase exponentially with temperature (20-80 °C). The hot water (80 °C) tends to extract a larger amount of water extractable organic C (WEOC) and N (WEON) than cold water (Zsolnay, 1996; Chantigny, 2003; Curtin et al., 2006; Ros et al., 2009). However, the composition of WEOM extracted by different temperatures is not well known. The published studies mostly focus on elemental ratios (C/N ratio) and few on molecular composition of cold and hot WEOM. The information on C/N ratios of WEOM is often contradictory. Some show that the C/N ratios in hot WEOM are narrower than cold WEOM and whole soil (Kaiser and Zech, 2000;

Greogrich et al., 2003; Curtin et al., 2006), but others report that the C/N ratios in hot WEOM are wider than or similar to cold WEOM and whole soil (Changtiny et al., 2010; Landgraft et al., 2006). To the best of my knowledge, only one study focuses on molecular composition of cold and hot WEOM and shows that hot WEOM contains more carbohydrates, phenols and lignin monomers, and organic N compounds than the cold WEOM in forest O horizon soils (Landgraft et al., 2006). The authors also suggested a significantly greater microbial contribution to hot WEOM than to cold WEOM, and hence they conclude that hot WEOM is more easily bioavailable than cold WEOM (Landgraft et al., 2006). Greogorich et al. (2003) also reported that hot WEOM has a greater biodegradability than cold WEOM in maize-cropped soil. However, other people argue that high extraction temperature increases the solubilization of recalcitrant compounds and are subsequently related to an increase in stable extractable organic matter such as nitrogen (Michrina et al., 1982; Matsumoto and Ae, 2004), indicating less biodegradability of hot WEOM. Balaria et al. (2009) estimated that microbial biomass can account for not more than 40% of the C extracted by hot water in forest organic horizons. More information on difference of cold and hot WEOM in their concentration, chemical composition, and biodegradability is currently needed.

Fluorescence spectroscopy provides a promising tool to characterize soil WEOM for its chemical molecular and functional properties. It produces fluorescence excitation-emission matrix (EEM), which, using parallel factor analysis (PARAFAC), can be decomposed into chemically meaningful components (eg. humic-like, fulvic-like, protein-like including tryptophan-like and tyrosine-like components). The concentration

of each component can be approximately estimated by its percentage of total fluorescence intensity of WEOM (relative loading). The biodegradable dissolved organic C is positively correlated with protein-like components but negatively correlated with humic-like components (Fellman et al., 2008). Several other indexes can be derived from fluorescence EEMs. First, fluorescence intensity at excitation 254 nm wavelength and emission 300-480 nm can provide information as to the degree of humification of WEOM by the humification index (HIX) (Zsolnay et al., 1999). High HIX usually indicates that organic matter is chemically complex and of condensed (aromatic) nature, resulting in difficulty of microbes to use these organic compounds. Hence, the HIX is negatively correlated with biodegradability of WEOC (Kalbitz et al., 2003; Bu et al., 2010). Second, fluorescence spectroscopy can be used to calculate fluorescence efficiency index (F_{eff}). The F_{eff} is proportional to the fluorophores quantum efficiency (Zsolnay, 2003) and negatively correlated with the size of organic molecules (Ewald et al., 1988) due to internal quenching in higher weight molecules (Bayer et al., 2002). Third, fluorescence index derived from fluorescence spectroscopy can be used to differentiate microbial derived from terrestrial or plant derived organic matter sources (McKnight et al., 2001). The FIX value ranges 1.7-2.0 for microbial derived organic matter and 1.4-1.5 for terrestrially or plant derived organic matter (McKnight et al., 2001). Additional spectroscopic index commonly used to indicate aromaticity of organic matter is specific ultraviolet absorbance (SUV_{254}), which has been reported to negatively correlate with the biodegradability of organic matter (Kalbitz et al., 2003; Saadi et al., 2006). A similar index of SUV_{280} also is used to indicate the aromaticity and molecular

weight of organic matter (Chin et al., 1994). The biodegradability of hot WEOC increases with decreasing aromaticity and humic indices (Bu et al., 2010).

The objectives of the present study were: 1) to compare the concentration, chemical characteristics, and biodegradability of water extractable organic matter extracted at room temperature (22 °C) and 80 °C; and, 2) to explain biodegradability of cold and hot WEOM with their respective chemical characteristics. Specifically, I hypothesized that: 1) hot water extracts higher amount of WEOC and WEON; and, 2) the hot water extracts more microbial-derived organic matter than cold water, resulting in an increase of FIX and protein-like component in hot WEOM than cold WEOM, and an decrease of F_{eff}, SUV_{254} and HIX in hot WEOM than cold WEOM; 3) the biodegradability of hot or cold WEOM is positively correlated with protein-like components but negatively correlated with SUV_{254}, HIX, and F_{eff}.

4.2 Materials and methods

4.2.1 Soils

Fourteen soil samples were collected at major agricultural areas (Fairbanks, Delta Junction, and Palmer) of subarctic Alaska, USA. Among the 14 samples, nine soil samples (0-10 cm) was taken from forest, conservation reserve program (CRP), and agricultural land in Delta Junction, AK, USA (145°20'W and 63°55'N) in 2005. Forest lands are dominated by the native coniferous forest such as blank spruce (*Picea mariana*) and a thick layer of feather mosses on the forest floors. Agricultural lands were converted from part of the native forest in 1978. Since then, the agricultural land was under continuous barley (*Hordeum valgare L.*) with conventional tillage (disked each spring).

Conservation Reserve Program (CPR) land grows a mixture of shrubs, forbs, and grasses. It was originally from part of the agricultural land but was converted to CRP in 1992. No lime was applied in all land uses. The remaining soils of the 14 samples were taken from pervious experiment in 2007. These samples were individually taken from 0-15 cm soil surface from the Fairbanks Experiment Farm (145°20'W and 65°49'N), Matanuska Experiment Farm at Palmer (149°22'W and 61°37'N), Delta Junction Field Research Site (151°30'W and 65°44'N), and Fairbanks Rosie Creek Organic Farm (148°50'W and 64°45'N). All 14 samples were air dried and sieved (<2 mm) before analysis for pH, total C and N. Soil pH was determined in a 1:1 soil/water suspension using a soil pH meter 140 (Corning Inc., New York, USA). Soil texture was analyzed by the hydrometer method after dispersion with sodium hexametaphosphate. Soil total C and N was measured by thermal combustion analysis using a LECO Truspec CN analyzer (LECO Corporation., St. Joseph MI, USA).

4.2.2 Sequential extraction of cold and hot WEOM

The extraction of cold and hot WEOM followed the procedure used by Curtin et al. (2006). Soil samples with three replicates were used for cold WEOM extractions. DI water (DI water, 90 ml) was added respectively to soil samples (12 g) in 250 ml Nalgene plastic bottles. The bottles were shaken for 30 min on an Eberbach 6000 reciprocal shaker (Eberbach Corporation, Michigan, USA) at room temperature (22 ±1 °C). Then, they were centrifuged at 900 x g for 30 min with an Eppendorf 5810R centrifuge (Eppendorf North America Inc., NewYork, USA). After centrifugation, the supernatant

was filtered through 0.45 μm filters. After filtration, 90 ml of the filtrate was transferred to glass flasks for cold WEOM incubation.

To obtain hot WEOM, the centrifuge tube plus soil residues used for cold WEOM extraction were weighed to calculate the entrained water volume. Another 90 mL denioized water was added into soil residues and the tubes were placed in a hot water bath at 80 °C for 16 h. The tubes were then centrifuged. The solution was then filtered through 0.45 μm (GN-6, Pall Corporation, New York, USA) and transferred to glass flasks for hot WEOM incubation.

A control of pure DI water with 3 replicates was set up for both cold and hot water extraction.

4.2.3 Inoculation

An inoculum was prepared for incubation of cold and hot extractions using the 14 soils. All air-dried soils were rewetted to 60% of water holding capacity and incubated for 2 weeks at 15 °C to reactivate the microorganisms. The preparation mostly followed the procedure used by Kalbitz et al. (2003). Each of 14 soils was extracted by a 4 mM $CaCl_2$ solution with a soil:solution ratios of 1:2 (w/v). The suspension was shaken for 15 min before filtering through a 3 μm filter paper (Whatman No.44 Ashless Filter, Whatman International Ltd, England) to remove large particles and most of grazing microfauna. After filtration, the solution extracted from each soil was mixed with an equal volume so that a representative inoculum was obtained.

4.2.4 Incubation of cold and hot WEOM

McDowell et al. (2006) suggested that a 7- day incubation at room temperature 20 °C can obtain relatively labile DOC incubation and a 42-d incubation can determine labile and relatively refractory components. In our study, soil solution was incubated at a relatively low temperature of 15 °C. The mineralization rate can be lower at 15 °C than 20 °C. Hence, we chose a 21-d incubation to make sure that it captures most of the labile WEOC and WEON and little of refractory component of WEOC and WEON.

The incubation for cold and hot WEOM was carried out following a method used by Kalbitz et al. (2003). Inoculum (1 ml) was added into a 500 mL glass flask containing cold or hot WEOM (90 ml). To provide extra surface area for microbial growth, glass microfiber filters 691 (VWR) were cut into 0.5 cm × 0.5 cm pieces and three pieces were added to each flask. During the incubation, no nutrients were added in solution. The flask was covered with a plastic cap loosely to allow air movement. The incubation was carried out in dark at 15 °C for 21 days. To allow adequate aeration, the flask was gently shaken by hand every day during the incubation. At the end of incubation, about 3 ml solution had evaporated and the solution in flask was set to original volume (90 ml) using DI water. The solutions were then filtered through 0.45 μm filters and kept in refrigerate at 4 °C until further analysis. The concentration of WEOC and WEON was determined before and after incubation. The proportional loss of WEOC and WEON during the 21-day incubation was calculated. In this study, all results were calculated based on oven dry soil weight (105 °C for 24 hrs).

4.2.5. Analyses

4.2.5.1 C and N analyses

Soil cold and hot water extraction before and after incubation were used for C and N analysis. Concentrations of inorganic C, total C and N in extractions were determined using a Shimadzu TOC-V Organic Carbon and Total Nitrogen Analyzer (Shimadzu Scientific Instruments Inc., Columbia, USA). In our soils, inorganic C was negligible (around 0.88 mg L^{-1}). Hence, the total C in cold and hot water extractions reported was referenced to cold or hot WEOC. Ammonium (NH_4^+-N) and nitrate (NO_3^--N) in extractions were measured using continuous flow analysis technique on an Alpkem Rapid Flow Analyzer (Astoria-Pacific International, Oregon, USA). The concentration of cold and hot WEON was calculated by subtracting mineral (NH_4^+-N and NO_3^--N) N from total N in water extraction.

4.2.5.2 UV measurement

The cold and hot water extractions before incubation were used for UV measurement. All the extractions were acidified to pH 2 by adding sufficient 1 *M* HCl to minimize the influence of pH on UV absorption. Absorbance at 254 nm (Abs_{254}) and 280 nm (Abs_{280}) was obtained in 1.0 cm quartz cuvette on a UV-VIS-NIR spectrophotometer UV-3600 (Shimadzu Scientific Instruments Inc., Columbia, USA). Absorbance (m^{-1}) was expressed as the ratio between optical density and optical length (0.01 m). The UV absorptivity at 254 or 280 nm (SUV_{254} or SUV_{280}, 1 mg C^{-1} m^{-1}) was calculated as (AbS_{254}/WEOC) × 100 or (AbS_{280}/WEOC) × 100.

4.2.5.3 Fluorescence spectroscopy and fluorescence derived indexes

The cold and hot water extraction before incubation were determined for their fluorescence EEM using a Fluoromax-4 spectrophotometer (Horiba Jobin Yvon Inc., New Jersey, USA) equipped with a 150-W ozone free xenon lamp as the excitation source. Before determination, in order to avoid inner filter effect, extractions were diluted by adding DI water so that the UV absorbance of extractions at 240 nm reached around 0.1 using a UV-VIS-NIR spectrophotometer UV-3600. The diluted samples were first used for the determination of original fluorescence EEMs with excitation wavelength of 240-400 nm and emission wavelength of 300-500 nm. The increment of excitation and emission wavelengths was 3 nm. The band width was 5 nm for excitation and emission slits and integration time was 0.1 s. The original EEMs of extractions were then corrected using files created by the manufacture (Horiba Jobin Yvon InC.) for instrument bias and Rayleigh scatter lines. The final EEMs of extractions were obtained by first subtracting the controls (average of three replicates) to remove Raman scatter effects, and then was normalized using the area from the DI water Raman peak at excitation wavelength 350 nm. In addition, fluorescence is physically impossible to occur in areas where the emission wavelength was less than the excitation wavelength. So, we used zero to replace the fluorescence intensity of this area.

Fluorescence intensities of cold or hot WEOM at excitation wavelength 254 nm and emission wavelength 300 - 480 nm were used to calculate their HIX values using the equation below (Zsolnay et al., 1999)

$$HIX = \sum I_{435\rightarrow480} / \sum I_{300\rightarrow345},$$

where HIX is the humification index, $\sum I_{300\rightarrow345}$ (the low quartile, LQ), is the sum of fluorescence intensity at emission wavelength from 300 to 345 nm, $\sum I_{435\rightarrow480}$ (the upper quartile, HQ), is the sum of fluorescence intensity at emission wavelength from 435 to 480 nm.

Fluorescence efficiency index (F_{eff}) was calculated as the ratio of maximum fluorescence intensity of each emission spectrum over the UV absorption at 254 nm (Zsolnay, 2003).

Fluorescence index (FIX) was calculated as the ratio of emission intensity at 450 nm to that at 500 nm for an excitation wavelength of 370 nm (McKnight et al., 2001).

4.2.6 Data analysis

All data in this study was analyzed using Matlab (Version 7.1, The MathWorks, Inc., Massachusetts, USA).

The final fluorescence EEMs of cold and hot WEOM were separated for parallel factor analysis (PARAFAC). The PARAFAC analysis was conducted using Nway Toolbox in the Matlab. During the analysis, a non-negativity constraint was applied to restrict negative fluorescence intensities that were chemically impossible. Two to nine components were fitted to the data to investigate the correct number of components. Core consistency (close to 100% is optimal) followed by split half analysis was used to assess the proper number of components for PARAFAC models (Bro and Kiers, 2003; Fellman et al., 2008). Contour plots and fluorescence intensity of each component was produced by PARAFAC model. The relative loading of each component was calculated as the percentage of total components.

One way analysis of variance (ANOVA) followed by a least significance difference (LSD) test was used to determine whether there was significant difference at probability of 0.05 in fluorescence components, HIX, LQ, HQ, SUV_{254}, SUV_{280}, F_{eff}, FIX between the cold and hot WEOM, or among land uses (forest, CRP, agricultural soils). Pearson correlation analyses and regression analyses were used to determine the association between the optical properties (fluorescence components, HIX, LQ, HQ, SUV_{254}, SUV_{280}, F_{eff}, FIX) and loss of WEOC and WEON in cold and hot WEOM.

4.3 Results

4.3.1 Soil basic properties, organic C and N extracted by cold and hot water

In this study, soils were of loam to sandy loam texture, represented a wide range of pH from 5.0 - 6.9, soil total C content from 2.3% - 8.3%, and soil C/N ratios from 12 – 24 (Table 4-1).

The cold and hot WEOC concentrations ranged from 38.4-138.5 mg kg^{-1} and 127.7-784.6 mg kg^{-1}, respectively (Table 4-1). The cold and hot WEOC averaged 0.2% of soil total C and 0.8% of soil total organic C, respectively. The cold and hot WEON concentrations ranged from 1.2-16.8 mg kg^{-1} and 17.5-69.6 mg kg^{-1}, respectively. The cold and hot WEON represented 0.004% of soil total C and 0.02% of soil total N, respectively. The hot WEOC and WEON concentrations were significantly ($p<0.05$) higher than cold WEOC and WEON for all soils. On average, the concentration of hot WEOC was 3.9 times higher than that of cold WEOC and the concentration of hot WEON was 5.2 times higher than that of cold WEON in all soils. The C/N ratios were 13.4-26.5 for cold WEOM to 12.4-23.6 for hot WEOM, respectively. Land use had no

significant (p>0.05) influence on the hot WEOC, hot WEON, cold WEOC, and cold WEON concentrations.

4.3.2 Loss of WEOC and WEON

The loss of cold and hot WEOC during 21 d incubation ranged 4.05-22.03% and 8.18-30.21%, respectively (Table 4-2). The loss of cold and hot WEON during 21 d incubation ranged 4.30-74.89% and 36.01-68.75%, respectively. On average, the loss of cold WEOC and WEON was 14% and 53% for CRP, 10% and 13% for forest, and 12% and 60% for agricultural soils and the loss of hot WEOC and WEON was 14% and 56% for CRP, 10% and 53% for forest, and 12% and 63% for agricultural soils during a 21-d incubation. The loss of hot WEOC and WEON was higher than that of cold WEOC and WEON in soils.

4.3.3 Fluorescence spectroscopy and fluorescence derived indexes in cold and hot WEOM

4.3.3.1 Fluorescence EEMs and components decomposed by PARAFAC

An example of excitation-emission matrix plots of cold and hot WEOM under different land uses was shown in Fig. 4-1. The visual "peak-picking" method showed that all samples contained two similar broad peaks at Em 440 nm/Ex 240nm and Em 440nm/Ex 330nm. However, cold WEOM from agricultural soil has another two peaks at the short excitation wavelength (Em <240 nm/Ex <240nm; Em <240 nm/Ex 270nm). There was a long fluorescence chain area for all hot WEOM, which might indicate other peaks in this area. Since it is difficult to visually identify these peaks, we used PARAFAC for further analysis.

The core consistency diagnostic scores of PARAFAC analysis for models from one- to five- components were 100%, 97%, 82%, 42%, 19% for cold WEOM and 100%, 100%, 77%, 52%, 5% for hot WEOM. The core consistency was smaller than 5% for six- to nine- component models. The variance explained by models from one to five components was 99.1%, 99.5%, 99.7%, 99.8%, 99.8% for cold WEOM and 98.2%, 98.9%, 99.2%, 99.4%, 99.4% for hot WEOM. The drop off in core consistency from three-component model to four-component model for both cold and hot WEOM indicates that the spectral resolution using three-component model was sufficient in describing the components in samples and probably provided the most appropriate model for cold and hot WEOM EEMs data set. The three component model can explain as high as 99.7% and 99.2% variance of fluorescence EEMs for cold and hot WEOM, which was only a minor difference (0.1% for cold WEOM and 0.2% for hot WEOM) from that of four component model. A second examination of models using split half analysis also supported that three component models are optimal for cold and hot WEOM.

Three fluorophore components decomposed by PARAFAC analysis for cold and hot WEOM were shown in Fig. 4-2. The component 1 in cold WEOM and the component 2 in hot WEOM had similar broad peak (Em 474-480 nm/Ex 240 and 348 nm), which was typical terrestrial humic substance-like fluorophore (Chen et al., 2003; Ohno and Bro, 2006; Fellman et al., 2008). The component 2 in cold WEOM and the component 1 in hot WEOM were similar with a shorter emission wavelength (Em 400-440 nm) than humic-like, which is considered as fulvic acid-like component (He et al., 2006; Santín et al., 2009). It was also attributed to the marine or fresh water humic-like component

(Yamashita and Tanoue, 2004; Stedmon and Markager, 2005). This pattern also was observed in the water extracts of soil, plant biomass and animal manure (Ohno and Bro, 2006). The spectral contours of the component 3 showed different protein contours between cold and hot WEOM. Cold WEOM showed the strong emission in the 335 to 340 nm at the low excitation wavelength 240 nm which is assigned to tryptophan-like components (Chen et al., 2003; Yamashita et al., 2008). Hot WEOM displayed unknown fluorophores with a shorter emission wavelength (300 nm) at the lower excitation wavelength. According to operationally defined fluorophore locations by Chen et al. (2003), this component is most likely composed of tyrosine-like components.

The relative loadings of the three components for cold and hot WEOM in all soils are shown in Fig. 4-3. The cold and hot WEOM were well modeled by the first two components. An average of 94% and 89% of the fluorescence intensity was contributed by humic-like and fulvic-like components in cold and hot WEOM, respectively. However, the distribution of the first two components in cold and hot WEOM was markedly different, with the fulvic-like fluorophore being higher than humic-like fluorophore for the cold WEOM but lower than humic-like fluorophore in hot WEOM. The statistic analysis (Table 4-3) showed that increasing extraction temperature resulted in a significant ($p<0.05$) decrease in the contribution of fulvic-like fluorophore for forest and agricultural soils, a significant ($p<0.05$) increase in the humic-like fluorophore for all soils and in the protein-like fluorophore for CRP and agriculture soils. There was no significant ($p>0.05$) differences in relative loadings of the three components of cold and hot WEOM among land uses.

4.3.3.2 HIX, F_{eff}, FIX, SUV_{254} and SUV_{280} in cold and hot WEOM

For all soils, the hot WEOM had significantly ($p<0.01$) lower HIX, SUV_{254}, and SUV_{280}, but significantly higher ($p<0.01$) LQ, and F_{eff} than cold WEOM (Table 4-4). Cold and hot WEOM showed no significant ($p>0.05$) differences in FIX and HQ. There were no significant difference in SUV_{254}, F_{eff}, LQ, HQ, and HIX of cold and hot WEOM among forest, CRP, and agricultural land.

4.3.4. WEOM biodegradation in relation to WEOM characteristics

The loss of cold WEOC was significantly ($p<0.05$) increased with the decreased SUV ($r = -0.67$, $p<0.01$), whereas it increased with LQ ($r = 0.53$, $p<0.05$), the increased protein-like component loading ($r = 0.78$, $p<0.01$) (Table 4-5). The loss of hot WEOC was significantly ($p<0.05$) increased with the decreased HIX ($r = -0.73$, $p<0.01$), HQ ($r = -0.58$, $p<0.05$), fulvic-like components ($r = -0.64$, $p<0.01$), whereas it increased with protein-like component loading ($r = 0.78$, $p<0.01$).

4.4 Discussion

Hot water in soil tended to extract a significant higher amount of WEOC and WEON than cold water (Table 4-1). Increases of WEOC and WEON in hot water extractions than cold water extractions has been reported before (Zsolnay, 1996; Chantigny et al., 2010; Curtin et al., 2006; Ros et al., 2009), and this increase in soil may be attributed to released soluble organic C and N by depolymerization of soil organic matter, decreased viscosity of soil humus, physical state change of soil organic matter, and killed microbes (Chantigny et al., 2010).

We found the mass loss of cold WEOC from all soils was 4.05-22.03% of initial cold WEOC (Table 4-2). In forest soils, the biodegradability of cold WEOC averaged 9%, which lies near the low end range of the 7%-17% reported by Kalbtiz et al. (2003) in humified layer of spruce forest and by Kiikkilä et al. (2006) in the humus layer under coniferous forest (Norway spruce and Scots pine). In agricultural soils, 12% of the cold WEOC was lost, which was lower than 17% DOC loss by Kalbtiz et al. (2003) in humified layer of spruce forest and agricultural soil amended with mineral fertilizer. We also found that the loss of hot WEOC during 21 d incubation ranged 8.18-30.21% of the initial hot WEOC in all soils. In forest soils, biodegradability of hot WEOC averaged 12%, which was the lowest value in 12-20% of hot WEOC loss in coniferous forest, deciduous forest, and alpine meadow (Bu et al., 2010). The mass loss of hot WEOC was 25% for agriculture soils, which was much lower than a range of 64-78% hot WEOC loss over a 42-d incubation in maize-cropped agricultural soils (Gregorich et al., 2003). In sum, the loss of WEOC in this study was smaller than that reported in previous literature. Longer incubation times tend to result in greater net removal of dissolved organic C from solution and higher values for biodegradable dissolved organic C (McDowell et al., 2006). The low biodegradation in our study might be due to shorter incubation time (21 d) than that used (42 d or 90 d) by Greogorich et al. (2003) and Bu et al. (2010). Little biodegradability information was available in CRP soils. Here, we found an average of 14% cold WEOC loss and 24% for hot WEOC. As such, hot water extracted more labile C than did the cold water.

Previous study reported a higher biodegradation of WEON than WEOC in the humus layer under coniferous forest (Norway spruce and Scots pine) (Kiikkilä et al., 2006), and in maize-cropped agricultural soils (Gregorich et al., 2003). In this study, the loss of cold and hot WEON during 21 d incubation ranged 4.30-74.89% and 36.01-68.75%, respectively, which were significantly ($p<0.05$) higher than that of cold and hot WEOC (Table 4-2), suggesting that WEON was more biodegradable than WEOC. However, there was a poor correlation between loss of organic C and N (Table 4-5). In soil, the biodegradation is carried out by microbe which usually needs both C and N for energy and nutrient. In general, the mineralization of soluble organic C and N is linked. The poor correlation between loss of organic C and N may indicate that the soluble organic C or N might also be lost by other ways such as being filtered out due to forming the cloudy insoluble organic matter during the incubation.

Under different extraction temperatures, soil WEOM showed similar numbers of fluorophores including humic-like, fulvic-like, and protein-like components (Fig. 4-1 and Fig. 4-2). Among these three components, only the protein-like components were affected by extraction temperature. The protein-like component occurs as tryptophan-like fluorophore in cold WEOM but as tyrosine-like fluorophore in hot WEOM. In previous studies, the numbers and types of fluorophore components reported in cold WEOM varied depending on sources of organic matter. Ohno and Bro (2006) found five fluorophore components (humic-like, fulvic-like, and protein-like including tyrosine-like and trypotophan-like fluorophores) in cold WEOM of soil, wetland plants, manures, tree leaves, and crop tissue (Ohno and Bro, 2006). Zhang et al. (2011) reported three

fluorophore components (humic-like, fulvic-like, and tyrosine-like fluorophores) in cold WEOM from agricultural soils with different tillage and manure application. In this study, sources of cold WEOM were microbe or plant-derived organic matter under different land uses (CRP, forest, and agriculture). Apparently, different land uses did not contribute multiple fluorophore components in cold WEOM. Little information is available on fluorescence components of hot WEOM. This study indicated that hot water extraction did not increase numbers of fluorophore components than cold water. This similarity in numbers of fluorophore components in cold and hot WEOM may indicate that the source of organic matter was similar in cold and hot WEOM. In general, tryptophan-like fluorophore indicates the presence of intact proteins or less degraded peptide material, whereas the tyrosine-like fluorescence may be more degraded peptide material. The change of protein-like components from tryptophan-like to tyrosine-like fluorophore as extraction temperature increased may indicate the decomposition of intact protein when increasing extraction temperature. This result was consistent with an assumption of the partial hydrolysis of organic N and C in hot extractants (Gregorich et al., 2003; Chantigny et al., 2010).

Because the fluorophores identified from PARAFAC analysis were a mixture of many compounds, the true concentration of each fluorophore component can not be obtained for unknowing its fluorescence quantum efficiency. The relative loading of each component based on its fluorescence signal contributions only provided its relative concentrations. There was marked impact of extraction temperature on distribution of fluorophore components (Fig. 4-3. and Table 4-3). The sensitivity of the component

distribution to extraction temperature indicates that extraction temperature alters both the quantity and the chemical properties of soil WEOM. Fluorescence analysis showed that hot water extracted more humic acid-like and less fulvic acid-like than cold WEOM, which might reflect the presence of significant quantities of humic acid as extraction temperature increased. Curtin et al. (2006) also found that a large proportion of organic N extracted by hot water is resistant to acid hydrolysis, which is assumed as humic acid.

As extraction temperature increased, the humification index decreased because fluorescence emission was significantly ($p<0.05$) increased at the shorter wavelength region (LQ) but not at the longer wavelength (HQ) (Table 4-4). Hot WEOM also showed significantly ($p<0.05$) higher fluorescence efficiency but significantly ($p<0.05$) lower SUV_{254} and SUV_{280}. However, the fluorescence index was similar in cold and hot WEOM. Organic matter fluorescent at the LQ is of lower carbon/hydrogen (C/H) ratio (less humified) structure (Zsolnay et al., 1999). Hence, the increase of LQ and decrease of humification index in hot than cold WEOM here might indicate an accumulation of simple, less condensed and bioavailable organic matter. These less condensed and more bioavailable organic material might be released by microbial cell lysis (Turro, 1978). They also might be from transformation of non-fluorescencing substrate such as gluocose into strongly fluorescent material in the LQ (Akagi et al., 2007), which was evidenced by the great increase of fluorescence efficiency in hot than cold WEOM. The increase of fluorescence efficiency in hot than cold WEOM also might indicate that hot water extraction was dominated more by smaller weight molecules than cold water extraction. In general, high SUV_{254} or SUV_{280} values reflect high aromaticity of organic matter

(Fellman et al., 2008). Hot WEOM had lower SUV_{254} and SUV_{280} values than cold WEOM, indicating that hot WEOM was of less aromatic structure than cold WEOM. The FIX can differentiate the sources of organic matter with plant derived organic matter being lower in FIX than microbial derived organic matter (McKnight et al., 2001). In this study, a similar result in cold and hot WEOM may indicate that cold and hot WEOM were derived from similar sources of microbial and terrestrial organic matter.

Easily degradable organic matter is composed of less humified small molecules (Qualls and Haines, 1992), or N-enriched compounds such as amino acids, amino sugars, peptides, and proteins (Aiken and Leenheer, 1993). So, biodegradability of cold WEOC was positively correlated with protein-like fluorophore and LQ (Table 4-5). The biodegradability of hot WEOC was also closely correlated with protein-like fluorophore (Table 4-5). The recalcitrant fraction of organic matter usually is of higher aromaticity, more complex molecules or high molecular weight. So, the biodegradation of organic matter is often found to be negatively correlated with HIX, HQ, or SUV_{254} (Bu et al., 2010; Fellman et al., 2008; McDowell et al., 2006). Similarly, we also found a negative correlation between loss of cold WEOC and SUV_{254} and HIX, between hot WEOC loss and HIX, and HQ.

4.5 Conclusions

In this study, the loss of WEOC and WEON in hot extractions was higher than cold extractions during 21 d incubation. The analysis of fluorescence components, HIX, SUV_{254}, SUV_{280}, and F_{eff} showed that hot WEOM was of higher protein-like fluorophore, less humified, less aromatic, small weight molecules than cold WEOM, indicating hot

WEOM was of more bioavailable and labile than cold WEOM. However, our study also showed that the protein-like fluorophore was in the form of tryptophan-like fluorophore in cold WEOM but in the form of tyrosine-like in hot WEOM, indicating the hydrolysis of organic C and N in hot water extraction process. The bioavailable and labile organic matter in hot WEOM might be a product of heat hydrolysis or transformation induced by high temperature. In natural condition, it still needs to be verified if these labile or bioavailable organic matter can be released into the soil solution.

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Table 4-1. Soil basic properties and water extractable organic C (WEOC) and N (WEON) extracted by cold (22 °C) and hot (80 °C) water from different land use soils in Alaska

Soils	Location	Soil texture	Soil pH_{H2O}[1]	TC[2] %	Soil C/N	Cold WEOC mg kg^{-1}	Hot WEOC mg kg^{-1}	Cold WEON mg kg^{-1}	Hot WEON mg kg^{-1}	Cold water C/N	Hot water C/N
CRP											
1	Delta Junction	Sandy loam	5.4	4.5	22.5	70.9	262.2	3.9	33.2	18.3	7.9
2	Delta Junction	Sandy loam	5.4	4.1	20.5	109.6	205.6	5.3	34.1	20.5	6.0
3	Delta Junction	Loam	5.0	5.0	16.7	80.8	486.7	13.0	53.9	6.2	9.0
Mean			5.3	4.5	19.9	87.1 b	318.2 a	7.4 b	40.4 a	15.0	7.7
Forest											
4	Delta Junction	Sandy loam	5.2	4.7	23.5	71.6	302.6	1.9	30.0	37.8	10.1
5	Delta Junction	Loam	5.4	4.8	24.0	138.5	204.8	4.6	25.2	30.1	8.1
6	Delta Junction	Loam	5.4	4.8	24.0	70.7	484.4	1.2	27.1	60.6	17.9
Mean			5.3	4.8	23.8	93.6 b	330.6 a	2.6 b	27.4 a	42.8	12.0
Agriculture											
7	Palmer	Sandy loam	5.4	5.7	19.0	135.2	519.0	16.3	38.8	8.3	13.4
8	Delta Junction	Loam	5.4	3.8	19.0	65.6	784.6	6.4	69.6	10.2	11.3
9	Fairbanks	Loam	5.3	8.3	16.6	38.4	309.7	6.2	43.3	6.2	7.1
10	Fairbanks	Loam	5.3	3.1	15.7	81.9	423.2	9.5	64.8	8.6	6.5
11	Fairbanks	Loam	5.1	3.3	19.4	57.6	188.8	4.4	17.5	13.1	10.8
12	Delta Junction	Loam	6.9	2.3	12.0	101.4	127.7	16.8	29.9	6.0	4.3
13	Delta Junction	Loam	6.2	2.9	15.4	103.6	284.3	4.4	47.2	23.7	6.0
14	Delta Junction	Sandy loam	6.7	3.6	18.1	104.4	258.8	12.6	43.4	8.3	6.0
Mean			5.6	4.3	19.2	86.0 b	362.0 a	9.6 b	44.3 a	10.6	8.2

Different letters indicate significance at $p<0.05$ between cold and hot WEOM for each chemical characteristics.

[1] DI water (solution:soil=1:1 (v:w)).

[2] Total carbon.

Table 4-2. The loss of cold (22 °C) and hot (80 °C) water extraction C (WEOC) and N (WEON) during a 21-d incubation at 15 °C for Alaska soils

Soils	%WEOC loss		%WEON loss	
	Cold	Hot	Cold	Hot
1	14.17	30.21	54.46	55.74
2	12.28	23.04	51.79	64.95
3	14.85	19.33	52.43	48.03
Mean for CRP	13.77	24.19	52.89	56.24
4	4.15	14.17	23.18	59.41
5	13.29	9.91	4.30	61.35
6	10.96	12.24	10.63	36.01
Mean for forest	9.47	12.10	12.70	52.26
7	12.60	28.11	40.78	37.79
8	10.41	29.77	53.23	59.79
9	4.05	15.79	46.29	68.75
10	22.03	60.73	74.89	80.29
11	9.13	14.67	81.43	63.64
12	7.17	22.31	88.91	66.69
13	12.06	8.18	26.14	63.06
14	15.19	19.37	64.97	64.84
Mean for agriculture	11.58	24.87	59.58	63.11
Significance of land use	NS[1]	NS	Forest vs. CRP and agriculture	NS

[1] No significant difference ($p<0.05$) among land uses for each index.

Table 4-3. Land use and extraction temperature effects on relative loadings of the three components in the parallel analysis (PARAFAC) model for the water extractable organic matter

Soils	%Fulvic-like		%Humic-like		%Protein-like	
	Cold	Hot	Cold	Hot	Cold	Hot
1	48.22	32.77	44.02	57.30	7.75	9.93
2	49.22	35.04	44.07	54.05	6.70	10.91
3	46.78	36.53	44.11	52.78	9.11	10.69
Mean for CRP	48.07	34.78	44.07 b	54.71 a	7.86 b	10.51 a
4	57.03	37.97	38.69	50.30	4.28	11.73
5	49.98	35.98	40.82	54.49	9.20	9.53
6	52.89	37.38	41.62	53.05	5.49	9.56
Mean for forest	53.30 a	37.11 b	40.37 b	52.61 a	6.32	10.27
7	48.10	34.27	43.12	53.61	8.79	12.12
8	52.13	35.00	42.60	55.70	5.26	9.30
9	56.48	38.93	39.25	51.25	4.27	9.82
10	53.72	43.98	32.82	39.80	13.45	16.22
11	48.97	36.71	48.05	55.55	2.98	7.74
12	50.19	37.23	46.82	51.36	2.99	11.41
13	51.88	37.30	45.89	52.91	2.23	9.79
14	49.10	36.71	45.24	54.17	5.66	9.12
Mean for agriculture	51.32 a	37.52 b	42.97 b	51.79 a	5.70 b	10.69 a
Significance among land use	NS[1]	NS	NS	NS	NS	NS

Different letters indicate significance at $p<0.05$ between cold and hot WEOM for each land use.
[1]No significant difference ($p>0.05$) among land uses for each component in cold or hot WEOM.

Table 4-4. Land use and extraction temperature effects on chemical characteristics of water extractable organic matter (WEOM) in Alaska soils

Soils	LQ[1]		HQ[2]		HIX[3]		F_{eff}[4]		FIX[5]		SUV_{254}[6]		SUV_{280}[7]	
	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
1	0.06	0.23	1.19	1.14	19.61	5.58	0.08	0.48	1.07	1.07	1.90	0.31	0.76	0.38
2	0.16	0.32	1.63	1.48	12.90	4.77	0.08	0.50	1.05	1.05	1.65	0.65	1.50	0.36
3	0.22	0.27	1.42	1.46	9.81	5.41	0.07	0.93	1.11	1.09	1.57	0.29	1.80	0.30
Mean for CRP	0.15b	0.27a	1.41	1.36	14.10a	5.25b	0.08b	0.64a	1.08	1.07	1.71a	0.42b	1.35a	0.34b
4	0.08	0.26	1.43	1.26	18.66	4.92	0.04	0.62	1.13	1.10	3.51	0.41	2.51	0.15
5	0.20	0.21	1.67	1.38	9.78	6.60	0.08	0.33	1.06	1.08	1.77	0.70	1.03	0.19
6	0.10	0.32	1.44	1.79	15.04	5.23	0.07	0.85	1.12	1.08	2.01	0.38	1.58	1.06
Mean for forest	0.12b	0.26a	1.52	1.48	14.50a	5.58b	0.07b	0.60a	1.10	1.09	2.43a	0.50b	1.71a	0.47b
7	0.12	0.21	1.57	1.17	15.06	5.51	0.08	0.45	1.09	1.05	1.59	0.47	1.44	0.17
8	0.12	0.23	1.78	1.51	15.40	6.16	0.06	0.91	1.09	1.07	2.44	0.26	2.17	0.57
9	0.11	0.25	1.58	1.46	14.30	5.83	0.09	0.59	1.13	1.10	2.38	0.42	1.69	0.39
10	0.19	0.37	1.17	1.08	6.07	2.94	0.09	0.33	1.14	1.12	1.35	1.10	1.00	0.12
11	0.05	0.30	1.53	2.02	36.04	6.97	0.10	0.12	1.12	1.13	1.80	2.42	1.28	0.30
12	0.05	0.37	1.32	1.65	28.05	4.92	0.11	0.31	1.10	1.12	1.46	1.20	1.06	0.39
13	0.06	0.28	1.66	1.69	30.78	6.01	0.08	0.20	1.11	1.12	2.16	1.42	1.51	0.50
14	0.10	0.32	1.43	1.77	14.23	5.61	0.07	0.94	1.13	1.10	2.08	0.34	1.46	0.64
Mean for agr.[8]	0.07b	0.32a	1.47	1.70	24.35a	5.51b	0.09b	0.48a	1.11	1.11	1.90a	0.99b	1.34a	0.51b
Sign. (Land use)[9]	NS[10]		NS		NS		NS		NS		NS		NS	

Different letters indicate significance at $p<0.05$ between cold (22 °C) and hot (80 °C) WEOM for chemical characteristics.
[1] Low quartile, sum of fluorescence intensity across 300 - 345 nm. [2] High quartile, sum of fluorescence intensity across 435 - 480 nm. [3] Humification index. [4] Fluorescence efficiency. [5] Fluorescence index. [6] UV absorptivity at 254 nm. [7] UV absorptivity at 280 nm. [8] Agriculture. [9] significance among land use. [10] No significant difference ($p>0.05$) among land uses for each index.

Table 4-5. Pearson correlation between chemical characteristics and loss of cold (22 °C) and hot (80 °C) water extractable organic C (WEOC) and N (WEON) in Alaska soils

	Cold water		Hot water	
Indexes	%WEOC loss	%WEON loss	%WEOC loss	%WEON loss
Cold WEOC	1.00			
Cold WEON	0.15	1.00		
Hot WEOC	0.63*	0.51	1.00	
Hot WEON	0.11	0.48	0.36	1.00
pH	-0.04	-0.32	-0.16	0.30
SUV_{254}[1]	-0.67**	-0.47	-0.06	0.38
SUV_{280}[2]	-0.51	-0.31	-0.33	-0.38
F_{eff}[3]	0.12	0.56	-0.04	-0.38
FIX[4]	-0.12	0.19	0.01	0.56*
HIX[5]	-0.45	0.28	-0.73**	-0.28
LQ[6]	0.53*	-0.18	0.31	0.41
HQ[7]	-0.36	-0.42	-0.58*	-0.04
%Fulvic-like	-0.45	-0.27	-0.64**	-0.48
%Humic-like	-0.26	0.23	0.43	0.55*
%Protein-like	0.78**	-0.02	0.78**	0.28

n = 14.
*Significance at $p<0.05$.
**Significance at $p<0.01$.
[1] UV absorptivity at 254 nm.
[2] UV absorptivity at 280 nm.
[3] Fluorescence efficiency.
[4] Fluorescence index.
[5] Humification index = HQ/LQ.
[6] Low quartile, sum of fluorescence intensity across 300 - 345 nm.
[7] High quartile, sum of fluorescence intensity across 435 - 480 nm.

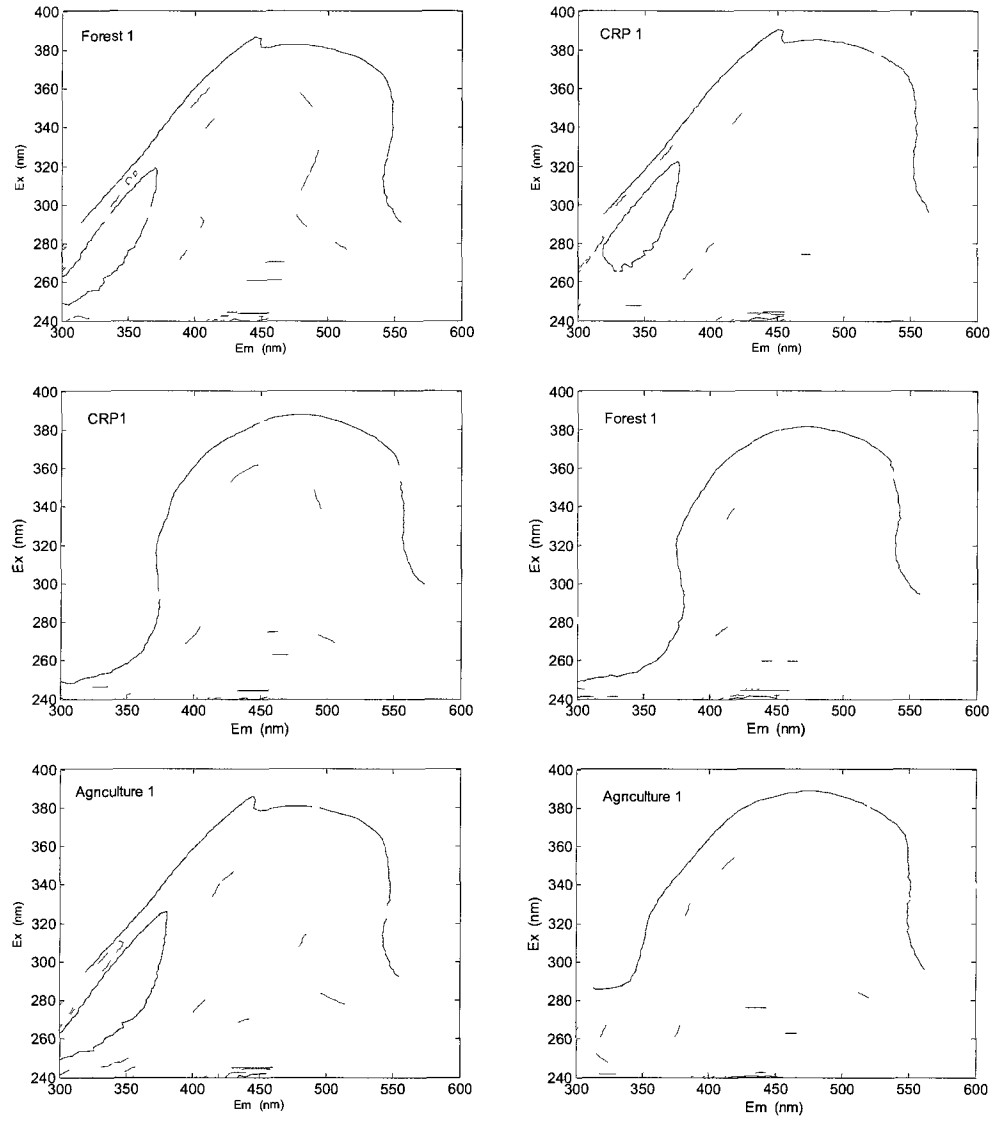

Fig. 4-1. The EEM fluorescence spectrum for cold water extractable organic matter (left) and hot water extractable organic matter (right) from sample soils of CRP, forest, and agriculture

Ex., excitation wavelength; Em., emission wavelength.

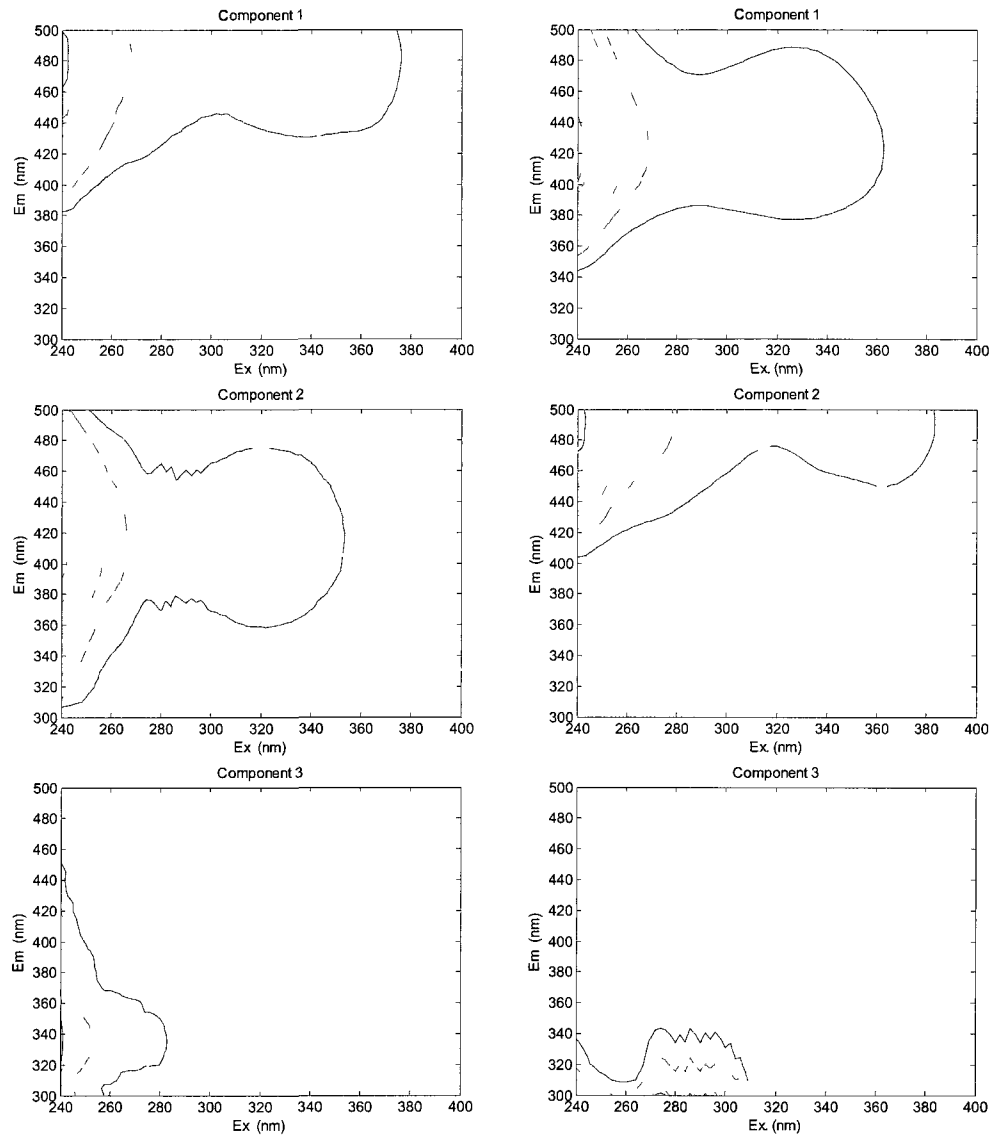

Fig. 4-2. The EEM spectral loadings of the three-component PARAFAC model of the cold (22 °C) water extractable organic matter (left) (Component 1, 2, and 3) and the hot (80 °C) water extractable organic matter (right) (Component 1, 2, and 3). Ex., excitation wavelength; Em., emission wavelength.

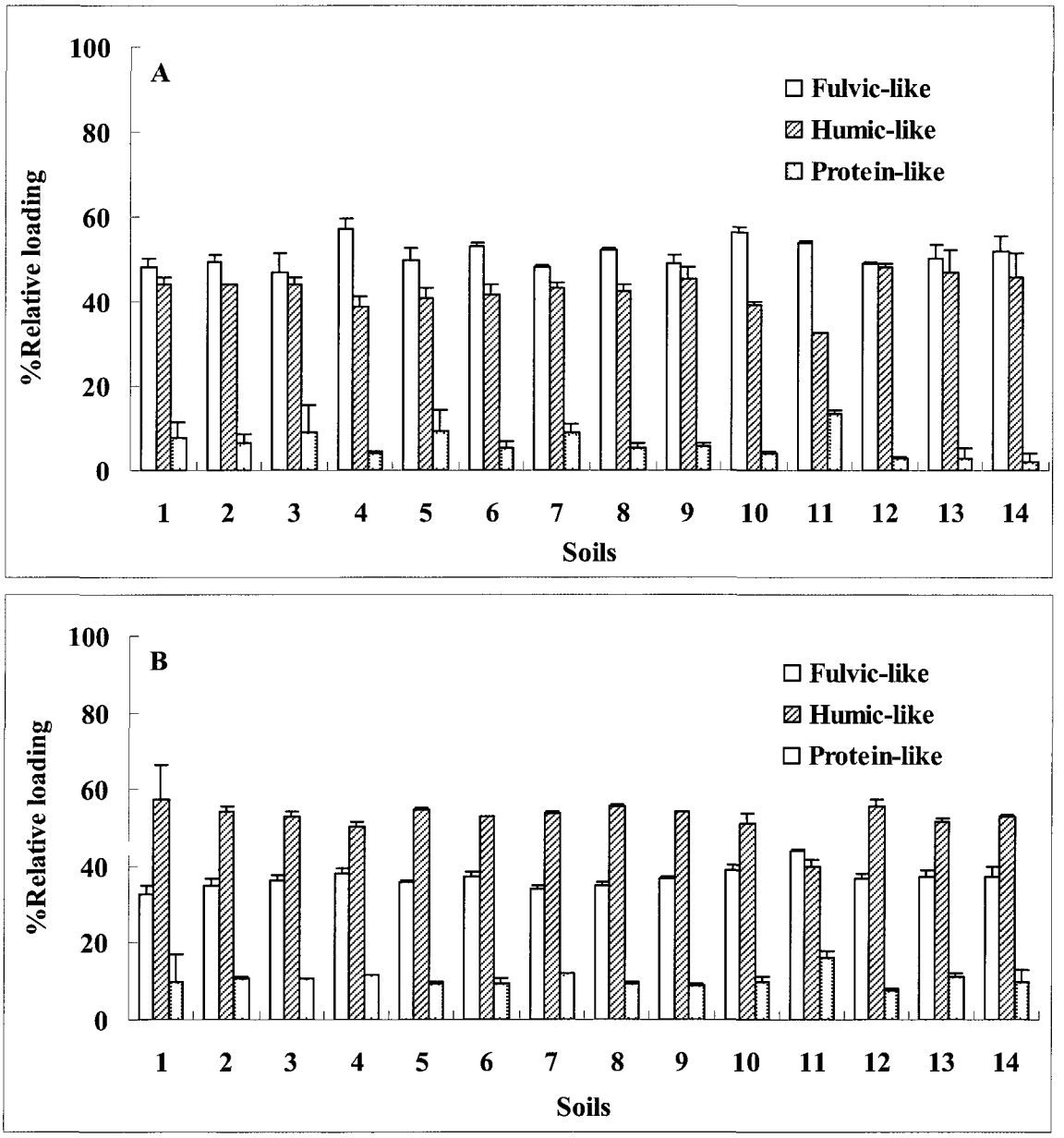

Fig. 4-3. Relative loadings of PARAFAC components of the cold (22 °C, A) and hot (80 °C, B) water extractable organic matter.

CHAPTER 5 PREDICTION OF SOIL NET NITROGEN MINERALIZATION WITH A DOUBLE EXPONENTIAL MODEL WITH EMPIRICAL RATE CONSTANT AND CHEMICALLY ESTIMATED POOL SIZES

Abstract

Kinetic models have been widely used to estimate soil N mineralization. To relate the kinetically determined N pools to soil properties is of theoretical as well as practical interest. This study aimed to correlate chemical or spectroscopic methods with model estimated pool sizes and to investigate the feasibility of using incubation, chemically, or spectroscopically estimated pool sizes for double exponential model to predict soil N mineralization in the field. The fixed rate constant double exponential models with empirical values of k_a = 0.693 weeks^{-1}and k_s = 0.051 weeks^{-1} were used to estimate active (N_a) and slow (N_s) organic N pool sizes for subarctic agricultural soils during a 24 weeks incubation under 15 °C and 55% water holding capacity. The N_s was linearly correlated with hot H_2O extractable organic N (R^2=0.75, p<0.05) or NaOH hydrolysable organic N (R^2=0.75, p<0.05). The N_a were linearly correlated with humification index (HIX) in cold water extraction (R^2=0.89, p<0.05). Soil N mineralization under field conditions using parameters estimated in laboratory and chemical methods fitted well with the measured data.

5.1. Introduction

An estimation of soil N mineralization is helpful in understanding the N dynamics and determining optimal fertilization rate in agricultural soils (Campbell et al., 1995). A simple double exponential model was proposed by Molina et al. (1980) and has been

widely used for this purpose (Molina et al., 1980; Deans et al., 1986; Hadas et al., 1983). The double exponential model assumes the potentially mineralizable organic N in soil can be divided into two different pools: an active pool (N_a) with active emineralization rate constant k_a and a slow pool (N_s) with slow mineralization rate constant k_s. It is described as $N_t = N_a (1-e^{-k_a t}) + N_s (1-e^{-k_s t})$, where N_t is the mineralized N at time t. In theory, these pools are of defined sizes that should not change with environmental conditions or with the procedure used to fit the models to the data. However, its usefulness to estimate soil N mineralization has been questioned by many researchers due to the following concerns:

1. The pool sizes are found to be inversely related to rate constants in the exponential models, indicating increasing one parameter can result in decreasing the other (Cabrera and Kissel, 1988; Bonde and Rosswall, 1987).
2. The estimated pool sizes and rate constants could be affected by the incubation time (Cabrera and Kissel, 1988; Dou et al., 1996). The changing pool sizes violates the general assumption that pool sizes are soil specific and are expected to be similar for a given soil.

Recently, Wang et al. (2004) solves these problems by fixing rate constant with experimental values $k_a = 0.693$ weeks^{-1} and $k_s = 0.051$ weeks^{-1}, while allowing the pools to vary to fit the data. This approach has been used to identify pools of mineralizable N in poultry litter added to soils (Cabrera et al., 2005).

The procedure that modifies double exponential model with fixed rate constant by Wang et al. (2004) provides a promising tool to estimate N mineralization in soils.

However, in Wang et al.'s method, N_a and N_s are different for each soil and its determination requires long-term incubation. To make them practical for use in routine soil tests, it is necessary to find a simple and fast method to estimate N_a and N_s. To the best of our knowledge, only one study has estimated N_a and N_s with a simple and fast method such as chemical extraction (Wang et al., 2004). It is reported that the size of N_a is correlated with the initial water-soluble organic N and microbial biomass C in soil while Ns is not correlated with any single organic C and N (Wang et al., 2004). Chemical methods extract soil organic matter portions based on their respective solubilities in various extractants (Coŭteaux et al., 2003; Kalbitz et al., 2003). They can not exclusively distinguish the labile organic matter from total soil organic matter (Bundy and Meisinger, 1994) and hence may only include some fractions of potentially easily mineralizable organic N. Wang et al. (2004) used the whole extracted organic matter to correlate with N_a and N_s but did not find the good chemical methods to predict N_a and N_s. The N_a or N_s is likely correlated with a fraction rather than the total amount of extracted organic matter. It is worth of a further examination of relationships between properties of chemically extracted organic matter and N_a or N_s in order to find good methods for estimating N_a or N_s.

The spectroscopic methods have been used to characterize water extractable organic matter (WEOM). Many studies have shown that biodegradation of soil organic matter is correlated with spectroscopic characteristics of water extracted organic matter (WEOM) such as UV absorptivity at 254 nm (SUV_{254}), humification index (HIX), fluorescence index (FIX), fluorescence protein-like or humic-like components (Bu et al., 2010;

Fellman et al., 2008). In general, the measurement of spectroscopic characterization is simple, quick, and highly sensitive. Also, spectroscopic characterization does not require complex sample preparation which can result in unnecessary artifacts (Zsolnay et al., 1999).

Therefore, the objectives of this study were to determine if it is possible to estimate N_a or N_s with chemical or spectroscopic methods and to determine the feasibility of using fixed double exponential model with chemical or spectroscopic methods estimated pool size to predict N mineralization dynamics under field conditions.

5.2 Materials and methods

5.2.1 Soil sampling

Eight soils were taken from major agricultural areas of Delta Junction, Fairbanks, and Palmer in Alaska, USA. Among these soils, three soil samples (0-15 cm) were taken from Delta Junction in 2005. The remaining five soil samples were from pervious experiment for other studies in 2007. These samples were individually taken from 0-15 cm soil surface from Fairbanks Experiment Farm (two samples), Matanuska Experiment Farm near Palmer (one sample), Rosie Creek Farm near Fairbanks (one sample), and Delta Junction Field Research Site (one sample). All of soil samples were air dried and sieved (<2 mm) before analysis of total C, N content, and pH. Soil pH was determined in a 1:1 soil/water suspension using a soil pH meter 140 (Corning Inc., New York, USA). Soil texture was analyzed by the hydrometer method after dispersion with sodium hexametaphosphate. Soil total C and N was measured by thermal combustion analysis

using a LECO Truspec CN analyzer (LECO Corporation., St. Joseph MI, USA). Properties of each soil were given in Table 5-1.

5.2.2 Methods to estimate potentially minealizable organic N

5.2.2.1 Chemical methods

5.2.2.1.1 Sequential extraction of cold (CWEON) and hot H_2O extractable organic N (HWEON)

The extraction procedure followed that used by Curtin et al. (2006). Soils were first used to extract the cold extractable organic N at room temperature (22 °C). After removing supernatant, the soil residue was again extracted by hot water (80 °C) to obtain hot water extractable organic N. Briefly, four grams of soil samples with 30 mL of deionized water were shaken in 50 mL centrifuge tubes for 30 min. The tubes were then centrifuged at 2600 rpm, followed by filtration through about 2.5 μm filter papers (Whatman No.42 Ashless Filter, Whatman International Ltd, England). The centrifuge tube plus wet soils was weighed to calculate the entrained water mass. Another 30 mL deionized water was added into tubes and tubes were placed in a hot water bath at 80 °C for 16 h. The tubes were then centrifuged and filtered as the extraction of hot water extractable N. Total extractable C and N in cold and hot water were determined using a Shimadzu TOC-V Organic Carbon and Total Nitrogen Analyzer (Shimadzu Scientific Instruments Inc., Columbia, USA). Extractable inorganic N (NH_4^+-N + NO_3^--N) in cold and hot water were measured using continuous flow analysis technique using an Alpkem Rapid Flow Analyzer. The extractable N in cold and hot water was calculated by the difference of total extractable N and inorganic N.

5.2.2.1.2 1 *M* NaOH extractable organic N

The analysis of 1 *M* NaOH hydrolyzable organic N was carried out following the procedure described by Wang and Li (1991). A soil sample (4.00 g) was weighed into a 200 mL wide mouth glass jar. Then, 10 mL 1 *M* NaOH was added to the jar with soil and a glass beaker with 10 mL H_3BO_3 indicator solution was placed into the jar. The glass jar was closed immediately with a lid fitted to the glass jar. The jar was incubated under 40 ℃ in an incubator for 24 h. The amount of NH_4^+-N released was determined by titration of the indicator with standard 0.0025 *M* H_2SO_4. The NaOH hydrolysable organic N was calculated by the difference of NH_4^+-N before and after NaOH treatment.

5.2.2.1.3 UV measurement at 260 nm in 0.01 *M* $NaHCO_3$ extraction ($NaHCO_3$_260)

The extraction of 0.01 *M* $NaHCO_3$ followed the procedure of Fox and Pierkielek (1978) and Hong et al. (1990). Briefly, 2.5 g of soil was shaken in 50 mL of 0.01 *M* $NaHCO_3$ for 15 min in a 50 mL centrifuge tube. The suspension passed about 2.5 μm filter paper and ultraviolet (UV) absorbance of the extract was measured at 260 nm with a UV-VIS-NIR spectrophotometer UV-3600 (Shimadzu Scientific Instruments Inc., Columbia, USA).

5.2.2.1.4 1 *M* HCl hydrolyzable organic N

The extraction of 1 *M* HCl followed the procedure modified by Xu et al. (1997). Ten grams of soil was weighed into a 70 mL digestion tube and mixed with 50 mL 1 *M* HCl. The tube was covered with small glass caps and heated on a block digester at 100°C. After 4 h, the tube was allowed to cool to room temperature and the suspension was filtered through about about 2.5 μm filter paper. A 20 mL aliquot of the filtrate was

pipetted into a 50 mL beaker, neutralized to pH 6.5 with 2 *M* NaOH, and transferred to a 100 mL volumetric flask and diluted with DI water. The NH_4^+-N was then analyzed using continuous flow analysis technique with the Alpkem Rapid Flow Analyzer. The HCl-hydrolyzable organic N was obtained by subtracting the initial NH_4^+-N from the total amount of NH_4^+-N after hydrolysis.

5.2.2.2 Spectroscopic methods

5.2.2.2.1 UV absorptivity at 254 nm (SUV_{254})

The SUV_{254} measurement was applied in cold and hot extractions. In order to avoid the influence of pH on UV absorption, all extractions were first acidified to pH 2 with 1 *M* HCl. Then, absorbance at 254 nm (Abs_{254}) was obtained in 1.0 cm quartz cuvette on a UV-VIS-NIR spectrophotometer UV-3600 (Shimadzu Scientific Instruments Inc., Columbia, USA). Absorbance (m^{-1}) is expressed as the ratio between optical density and optical length (0.01 m). The UV absorptivity (SUV_{254}, 1 mg C^{-1} m^{-1}) was calculated as (AbS_{254}/WEOC) × 100.

5.2.2.2.2 Fluorescence spectroscopic analyses and PARAFAC modeling

Fluorescence excitation emission matrix (EEM) of solution was measured using a Fluoromax-4 spectrophotometer (Horiba Jobin Yvon Inc., New Jersey, USA) equipped with a 150-W ozone free xenon lamp as the excitation source. Before determination, samples were diluted to avoid inner filter effect by adding deinoized water so that the UV light absorbance of solutions at 240 nm reached around 0.1 using a UV-VIS-NIR spectrophotometer UV-3600. The diluted samples were used for the determination of fluorescence EEMs. The excitation wavelengths ranged from 240 to 400 nm. The

emission wavelengths ranged from 300 to 500 nm. The increment of excitation and emission wavelengths was 3 nm. Excitation and emission slits were set at a 5 nm band width with an integration time 0.1 s. Using files created by the manufacture, sample EEMs were corrected for instrument bias and Rayleigh scatter lines were masked. The final EEMs for samples were obtained by first subtracting the controls (average of three replicates) to remove Raman scatter effects, and then was normalized using the area from the Milli-Q water Raman peak at excitation wavelength 350 nm. In addition, zero was set to the data points where the emission wavelength was less than the excitation wavelength, which is physically impossible.

Fluorescence EEMs were separated based on cold and hot WEOM. The separated EEMs were analyzed by PARAFAC models. Briefly, fluorescence EEMs were loaded into Matlab software (Version 7.1, The MathWorks, Inc., Massachusetts, USA). The PARAFAC analysis was conducted using Nway Toolbox in Matlab. A non-negativity constraint was applied to restrict negative fluorescence intensities that were chemically impossible. Two to nine components were fitted to the data to investigate the correct number of components. Core consistency (close to 100% is optimal) followed by split half analysis was used to assess the proper number of components for PARAFAC models (Bro and Kiers, 2003; Fellman et al., 2008). Contour plots and fluorescence intensity of each component was produced by PARAFAC model. The relative loading of each component was calculated as the percentage of total components.

In this study, core consistency diagnostic scores of PARAFAC analysis for models from one- to five- components were 100%, 97%, 82%, 42%, 19% fore cold WEOM and

100%, 100%, 77%, 52%, 5% for hot WEOM. The core consistency was smaller than 5% for six- to nine- component models. The variance explained by models from one to five components was 99.1%, 99.5%, 99.7%, 99.8%, 99.8% for cold WEOM and 98.2%, 98.9%, 99.2%, 99.4%, 99.4% for hot WEOM. The sharp drop off in core consistency from three-component model to four-component model for both cold and hot WEOM indicates that the spectral resolution using four-component model was much worse than that using three-component model and the three component model probably provided the most appropriate model for cold and hot WEOM EEMs data set.

Three fluorophores decomposed by PARAFAC analysis for cold and hot WEOM were shown in Table 5-2. According to previous studies listed in Table 5-2, these three fluorophores in cold and hot WEOM were classified into fulvic-like, humic-like, and protein-like.

5.2.2.2.3 Humification index (HIX)

Fluorescence intensities of cold and hot WEOM at excitation wavelength of 254 nm and emission wavelengths 300-480 nm were used to calculate the HIX values of these samples using equation (Zsolnay et al., 1999)

$$\text{HIX} = \sum I_{435\rightarrow480} / \sum I_{300\rightarrow345} \qquad (5.1),$$

where HIX is the humification index, $\sum I_{300\rightarrow345}$ (the low quartile, LQ), is the sum of emission fluorescence intensity from 300 to 345 nm, $\sum I_{435\rightarrow480}$ (the upper quartile, HQ), is the sum of emission fluorescence intensity from 435 to 480 nm.

5.2.2.2.4 Fluorescence index (FIX)

Fluorescence index of cold and hot WEOM was calculated as the ratio of emission intensity at 450 nm to that at 500 nm for an excitation wavelength of 370 nm (McKnight et al., 2001).

5.2.2.3 Laboratory incubation

Lab incubation was applied to all these soil samples. Each soil with three replicates was for incubation (a total number of 8×3) . Five grams of each sample was weighed into a 50 mL glass vial and added with distilled water (55% water holding capacity). The vials were incubated for 24 weeks at 15 °C. During the incubation, soil moisture content was monitored weekly and the caps of glass vials were loose in order to renew the atmosphere in the vials. Soil samples are destructively collected at 0, 1, 2, 4, 6, 14, 16, 20, and 24 weeks, followed by the extraction and determination of inorganic N (NH_4^+ + NO_3^--N). For its extraction, one gram of air dried samples was weighed out into centrifuge tubes and suspended in 10 mL 2 *M* KCl. The tubes were shaken for 30 min on a Eberbach 6000 reciprocal shaker (Eberbach Corporation, Michigan, USA) at room temperature (22 ± 1 °C). Then, the suspensions were extracted through Whatman No.42 (about 2.5 μm) filters. Inorganic N concentrations (NH_4^+-N + NO_3^--N) in extracted solutions were determined using continuous flow analysis technique sing an Alpkem Rapid Flow Analyzer (Astoria-Pacific International, Oregon, USA).

The inorganic N during the incubation was fitted by fixed double exponential model with fixed k_a value of 0.693 weeks^{-1} and k_s value of 0.051 weeks^{-1} (see Chapter 2).

$$N_t = N_a(1-e^{-0.693t}) + N_s(1-e^{-0.051t}) \qquad (5.2),$$

where two pools are used to describe the readily mineralizable N_a (mg kg^{-1}) and the less readily mineralizable N_s (mg kg^{-1}).

5.2.3 Effect of temperature and soil moisture on rate constant of N mineralization kinetic models

Two agricultural soils (soil 3 and 7) were randomly chosen for this goal. Five grams of soil was weighed into a 50 mL glass vial and suspended in distilled water (20%, 55%, and 90% water holding capacity). The vials were incubated for 24 weeks at 5, 15, 25 and 35°C. During the incubation, the caps of glass vials were loose in order to renew the atmosphere in the vials and soil moisture content was monitored weekly. Soil samples are destructively collected at 0, 7, 14, 28, 42, 84, 112, 84, and 168 days. One gram of collected samples was weighed out into centrifuge tubes and added with 10 ml 2 *M* KCl, followed by the determination of inorganic N by the procedure above in 5.2.2.3. The model 5.2 was used for the inorganic N data set at different temperature and soil moisture to estimate the rate constant k_a and k_s.

5.2.4 Field incubation

In a natural field environment, soil N mineralization may be affected by many factors such as dry-rewet event or residue addition. To minimize the complexity of soil N mineralization in natural field condition, we set up a controlled in situ incubation to test the feasibility of kinetic model to estimate N mineralization under fluctuating temperature and controlled soil moisture. Agricultural soils (60 g) were weighed into a 250 mL glass jars and moistured with distilled water (55% water holding capacity). The jars were loosely caped and placed in a bucket. A thermometer was placed in each bucket

and recorded every 1 hour with a data logger. The bucket was tightly covered with a lid and half buried into the ground at the Fairbanks Experiment Farm. During the incubation, soil moisture content was determined weekly by weight. Soil samples were destructively collected at 0, 14, 28, 84, and 168 days. Inorganic N (NH_4^+-N+NO_3^--N) was extracted with 2 *M* KCl and determined using the method described in 5.2.2.3.

The prediction of net N mineralization under field fluctuating temperature and soil moisture following the equation used by Wang et al. (2004):

$$N_{t1a} = N_a (1-e^{-k_{a1}t}) \quad (5.3)$$

$$N_{tia} = N_{t_{i-1}a} + (N_a - N_{t_{i-1}a})(1-e^{-k_{ai}t}) \quad i=2,3,4,\ldots \quad (5.4)$$

$$N_{t1s} = N_s (1-e^{-k_{s1}t}) \quad (5.5)$$

$$N_{tis} = N_{t_{i-1}s} + (N_s - N_{t_{i-1}s})(1-e^{-k_{si}t}) \quad i=2,3,4,\ldots \quad (5.6)$$

where N_{t1a} and N_{t1s} (mg N kg^{-1}soil) was the mineralized N from N_a and N_s during the first time interval; t was 1/7 weeks when daily time interval was used; N_{tia} and N_{tis} was the predicted cumulative amount of inorganic N produced from N_a and N_s by the end of the second time interval and so on for the following time intervals. The total N mineralized at time t was the sum of N_{t1a} and N_{t1s} for the first time interval and the sum of N_{tia} and N_{tis} for the second and the following time intervals.

5.2.5 Data analysis

All the analysis was done with Matlab (Version 7.1, The MathWorks Inc., Massachusetts, USA). Fixed double exponential models were fitted to all inorganic N data sets obtained at 15 °C and 55% soil water holding capacity. The widely used Marquardt algorithm and the iterative process were chosen to find the values of the

parameters of N_a and N_s. The N_a and N_s were assumed to be specific to soils. So, the estimated N_a and N_s were fixed when fixed double exponential models were used to study the effect of temperature and soil moisture on k_a and k_s. The interactive effect of soil moisture and temperature was correlated. Linear regression was also done to relate chemical methods, spectroscopic methods and model-estimated N_a, N_s, and N_a+N_s.

5.3 Results and discussion

5.3.1 Relationship between chemical, spectroscopic, and lab incubation estimated organic N

The N_a, N_s, and N_a+N_s were estimated from fixed double exponential models (Eq.2). They were related with chemically estimated organic N (Table 5-3). There was a significant linear relationship between N_a and $NaHCO_3$_260 (R^2=0.60, p=0.02), between N_s and HWEON (R^2=0.70, p=0.01), NaOH hydrolysable organic N (R^2=0.75, p=0.005), and HCl hydrolysable organic N (R^2=0.55, p=0.03), and between N_a+N_s and HWEON (R^2=0.74, p=0.006), NaOH hydrolysable organic N (R^2=0.77, p=0.004), and HCl hydrolysable organic N (R^2=0.59, p=0.03).

A further analysis of CWEOM and HWEOM showed that their spectroscopic properties were related with N_a, N_s, and N_a+N_s (Table 5-3). Significant linear relationships were found between Na and HIX in CWEOM (R^2=0.89, p=0.0004), protein-like in CWEOM (R^2=0.65, p=0.02), SUV_{254} in HWEOM (R^2=0.68, p=0.01), and FIX in HWEOM (R^2=0.78, p=0.003). The N_s was significantly linearly related with FIX in HWEOM (R^2=0.59, p=0.02). The total of N_a and N_s was significantly linearly related with FIX in HWEOM (R^2=0.73, p=0.004).

Among the chemical and spectroscopic methods, HIX in CWEOM was best for prediction of N_a because it can explain 89% (R^2=0.89) of the total variation in N_a while NaOH hydrolysable organic N was best for estimation of N_s and N_a+N_s because they explained 75% (R^2=0.75) and 77% (R^2=0.77) of the total variation in N_s and N_a+N_s, respectively.

Wang et al. (2004) reported that the prediction of N_a is primarily contributed by dissolved organic N (DON) or water extracted organic N (WEON), suggesting that the size of N_a is primarily determined by the amount of available substrate in soil before incubation. The available substrate could be derived from killed microbe during rewetting of air-dried soil (Davidson et al., 1987) or desorped soluble organic matter from mineral surfaces (Chantigny, 2003). However, there was a larger variation in the quality of soluble organic N in different soils (Wang et al., 2001). Not all soluble organic matter is equally susceptible to biological decay (Boyer and Groffman, 1996). In this study, we did not find a significant relationship between WEON (cold or hot) and N_a (p=0.09) but found that N_a was significantly correlated with spectroscopic properties in WEOM such as HIX and protein-like component in CWEOM, or SUV_{254} and FIX in HWEOM (p<0.05). These spectroscopic properties have been shown to highly correlate with the biodegradability of DOC or WEOC under different vegetation types or land uses (Bu et al., 2010; Fellman et al., 2008).

In the Wang et al. (2004) study, N_s was not correlated with any single organic N. Here, we found that there was a significant (p<0.05) correlation between N_s and NaOH hydrolysable organic N, HWEON, HCl hydrolysable organic N, and FIX in HWEOM,

indicating that these organic N measure the relatively resistant organic N in potentially mineralizable organic N pool. Since N_s averaged 70% of a total of N_a and N_s, the size of N_a+N_s could primarily affected by N_s size. Therefore, the size of N_a+N_s also had a good linear relationship with NaOH hydrolysable organic N, HWEON, HCl hydrolysable organic N, and FIX in HWEOM.

The good correlation between N_a or N_s and chemical or spectroscopic methods such as HIX in CWEOM, or NaOH hydrolysable organic N does not imply these methods were a direct measurement of N_a or N_s pool sizes. For example, the NaOH hydrolysable organic N size (90-158 mg kg^{-1}) was always larger than N_s size (15-86 mg kg^{-1}), indicating that N_s might only be a fraction of NaOH hydrolysable organic N.

5.3.2 Two methods to estimate N_a and N_s

In general, the pool sizes of soil organic matter are soil specific (Wang et al., 2004). During a-24 weeks soil incubation at 15 °C and 55% water holding capacity, the N_a and N_s estimated from fixed double exponential model (5.2) were 23.73 and 60.69 mg kg^{-1} for agricultural soil 3, and 22.97 and 67.87 mg kg^{-1} for agricultural soil 7, respectively.

Chemical and spectroscopic methods were also used to estimate N_a and N_s. Best linear relationship was found between N_a and humification index in cold water extraction (CHIX, R^2=0.89, p=0.0004), and between N_s and NaOH hydrolysable organic N (R^2=0.75, p=0.005) (Table 5-3). The linear relationship can be described with equation 7 and 8 as below:

$$N_a = 35.49 - 0.78\ CHIX \qquad (5.7)$$

$$N_s = -66.77 + 0.89\ SON_{NaOH} \qquad (5.8)$$

where CHIX is humification index of cold water extracted organic matter (dimensionless) and SON_{NaOH} is NaOH hydrolysable organic N (mg kg^{-1}). By the equation 5.7, N_a was estimated as 24.31 with 95% confidence interval of 16.3-32.3 mg kg^{-1} for agricultural soil 3 and 23.45 mg kg^{-1} with 95% confidence interval of 15.5-31.4 mg kg^{-1} for agricultural soil 7. By the equation 5.8, N_s was estimated as 73.81 mg kg^{-1} with 95% confidence interval of 53.3-94.3 mg kg^{-1} for agricultural 3, and 68.39 mg kg^{-1} with 95% confidence interval of 27.7-109.1 mg kg^{-1} for agricultural soil 7.

The 95% confidence intervals of chemically estimated N_a or N_s covered the values of model-estimated N_a and N_s, indicating the feasibility of chemical methods in estimating N_a and N_s. The quick chemical method for estimating N_a and N_s makes it possible to use N_a or N_s by soil testing laboratories. However, in this study, linear relationships between N_a or N_s and chemical methods were based on a limited soil sample size (n=8). As sample sizes increase, the linear equations may greatly change. In order to accurately estimate mineralizable organic N, the relationships between N_a or N_s and chemically-estimated organic N deserves an investigation in a wider range of soil samples in the future.

5.3.3 The k_a and k_s modified by soil moisture and temperature

The k_a and k_s were changing in a linear relationship with soil moisture under a given temperature for agricultural soil (Fig. 5-1). This linear relationship gave higher R^2 (>0.95) under 15 and 25 °C than 5 and 35 °C for both soils. The k_a and k_s were significantly affected by temperature (Fig. 5-1). The relationships between rate constant and

temperature can be described by the Q_{10} function. The interactive effect of soil moisture and temperature was correlated following the equations below:

Agricultural soil 3: $k_a=0.693\ f_\theta \times f_T = 0.693\ (0.21+0.56\times\theta/\theta_{max}) \times 1.47^{(T-15)/10}$ (5.9),

$R^2=0.91$,

$k_s=0.051\ f_\theta \times f_T = 0.051\ (0.098+0.51\times\theta/\theta_{max}) \times 1.83^{(T-15)/10}$ (5.10),

$R^2=0.95$,

Agricultural soil 7: $k_a=0.693\ f_\theta \times f_T = 0.693\ (0.03+0.39\times\theta/\theta_{max}) \times 2.25^{(T-15)/10}$ (5.11),

$R^2=0.92$,

$k_s=0.051\ f_\theta \times f_T = 0.051\ (0.02+0.22\times\theta/\theta_{max}) \times 2.51^{(T-15)/10}$ (5.12),

$R^2=0.97$,

where f_θ and f_T are moisture and temperature correlation factors, respectively; θ_{max} is the optimum soil moisture for net N mineralization.

Previous studies mostly found an exponential change of rate constant following the temperature, which can be described by Q_{10} (Wang et al., 2004). Similarly, we found that rate constant k_a and k_s followed exponential curves as temperature rises.

5.3.4 The N mineralization from 112 d field incubation

For the field incubation, the soil moisture was controlled within a range of 52%-55% water holding capacity (0.14-0.20 g g^{-1}soil for soil 3 and 0.16-0.22 g g^{-1}soil for soil 7) to avoid the effect of dramatic drying-rewetting and denitrification (Fig. 5-2B). Under the field condition, the air temperature fluctuated within a range of 1.1 and 32.8 °C (Fig. 5-2A). The predicted dynamics of inorganic N in agricultural soils during 112-d field incubation fitted measured data well (Fig. 5-2 C and D).

Wang et al. (2004) used fixed double exponential model to satisfactorily predict N mineralization dynamics of Palexeralf and Rhodoxeralf soils in 126-d field incubation under temperature fluctuation of 2.5-35.8 °C. Following the similar procedure of modifying double exponential model, we also predicted N mineralization well in subarctic 112 d field incubation under temperature fluctuation of 1.1-32.8 °C. Furthermore, we found that the model parameters N_a an N_s estimated by chemical methods also agreed well with the measured data in field incubation. However, attention should be paid to the fact that the prediction here was based on daily mean temperature. Studies have found that prediction based on daily mean temperature was lower than the measured data because mineralization rate constant increases nonlinearly increases with the increase in the Q_{10} and the amplitude of the temperature fluctuation (Wang et al., 2004; Sierra, 2002; Das et al., 1995).

5.4 Conclusions

During a 24-week incubation, N mineralization in agricultural soils were divided into easily mineralizable organic N pool (N_a) and relatively resistant organic N pool (N_s). The size of N_a and N_s could be best linearly related with the HIX of cold water extracted organic matter and NaOH hydrolysable organic N, respectively. The sizes of N_a and N_s were also estimated by double exponential models using empirically defined values for k_a and k_s. Estimated mineralizable N from both methods could match well with measured N mineralization in field incubation with fluctuating temperature and controlled soil moisture.

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Table 5-1. Properties of soils used in this study.

Soil	Location	Soil texture	pH[1]	%C	C/N
1	Delta Junction	Sandy loam	5.4	5.7	19.0
2	Delta Junction	Loam	5.4	3.8	19.0
3	Delta Junction	Loam	5.0	5.0	16.7
4	Palmer	Loam	5.3	3.1	15.7
5	Delta Junction	Loam	5.1	3.3	19.4
6	Fairbanks	Loam	6.9	2.3	12.0
7	Fairbanks	Loam	6.2	2.9	15.4
8	Fairbanks	Sandy loam	6.7	3.6	18.1

[1] pH in 1:1 soil/water suspension.

Table 5-2. Fluorescence components decomposed by parallel analysis (PARAFAC) in cold (CWEOM) and hot water extractions (HWEOM).

Components	Peaks		Fluorescence components
	CWEOM	HWEOM	
1	Em: 400-440 Ex: <240, 320-340	Em: 400-440 Ex: <240, 320-340	Fulvic-lik (Hernandez et al., 2007; Sierra et al., 2005)
2	Em: 460-500 Ex:<240, 340-360	Em: 480-500 Ex:<240, 340-360	Humic-like (Chen et al., 2003; Ohno and Bro, 2006; Fellman et al., 2008)
3	Em: 320-340 Ex: <240	Em: <300 Ex: <240, 270-280	Protein-like (Chen et al., 2003; Coble, 1996)

Em, emission wavelength (nm). Ex, excitation wavelength (nm).

Table 5-3. Linear regression of chemically-estimated and model-estimated organic N in subarctic agricultural soils (n=8).

Methods	N_a[1] mg N kg^{-1} soil		N_s[2] mg N kg^{-1} soil		N_a+N_s mg N kg^{-1} soil	
	R^2	*p*	R^2	*p*	R^2	*p*
Chemical methods						
HWEON[3],mg N kg^{-1} soil	0.45	0.07	0.70	0.01	0.74	0.006
CWEON[4],mg N kg^{-1} soil	0.42	0.09	0.35	0.14	0.42	0.09
NaOH hydrolysable N, mg N kg^{-1} soil	0.40	0.09	0.75	0.005	0.77	0.004
HCl hydrolysable N, mg N kg^{-1} soil	0.40	0.09	0.55	0.03	0.59	0.03
$NaHCO_3$_260[5], UV absorbance	0.60	0.02	0.28	0.19	0.40	0.10
Spectroscopic property in CWEOM[6]						
SUV_{254}[7], l mg C^{-1} m^{-1}	0.06	0.56	0.39	0.10	0.28	0.18
FIX[8]	0.02	0.87	0.19	0.21	0.18	0.20
HIX[9]	0.89	0.0004	0.12	0.40	0.17	0.31
Humic-like, %	0.50	0.05	0.07	0.54	0.05	0.61
Protein-like, %	0.65	0.02	0.03	0.68	0.03	0.68
Spectroscopic property in HWEOM[10]						
SUV_{254}, l mg C^{-1} m^{-1}	0.68	0.01	0.23	0.23	0.36	0.12
FIX	0.78	0.003	0.59	0.02	0.73	0.004
HIX	0.03	0.70	0.05	0.60	0.05	0.59
Humic-like, %	0.14	0.36	0.05	0.59	0.08	0.50
Protein-like, %	0.01	0.78	0.01	0.95	0.003	0.90

R^2 and *p*, determination coefficient and probability of linear regression.
[1] The labile organic N pool size estimated from fixed double exponential model.
[2] The resistant organic N pool size estimated from fixed double exponential model.
[3] Hot water extracted organic N.
[4] Cold water extracted organic N.
[5] UV absorbance of 0.01 *M* $NaHCO_3$ extraction at 260 nm.
[6] Cold water extractable organic matter.
[7] UV absorptivity at 254 nm.
[8] Fluorescence index.
[9] Humification index.
[10] Hot water extractable organic matter

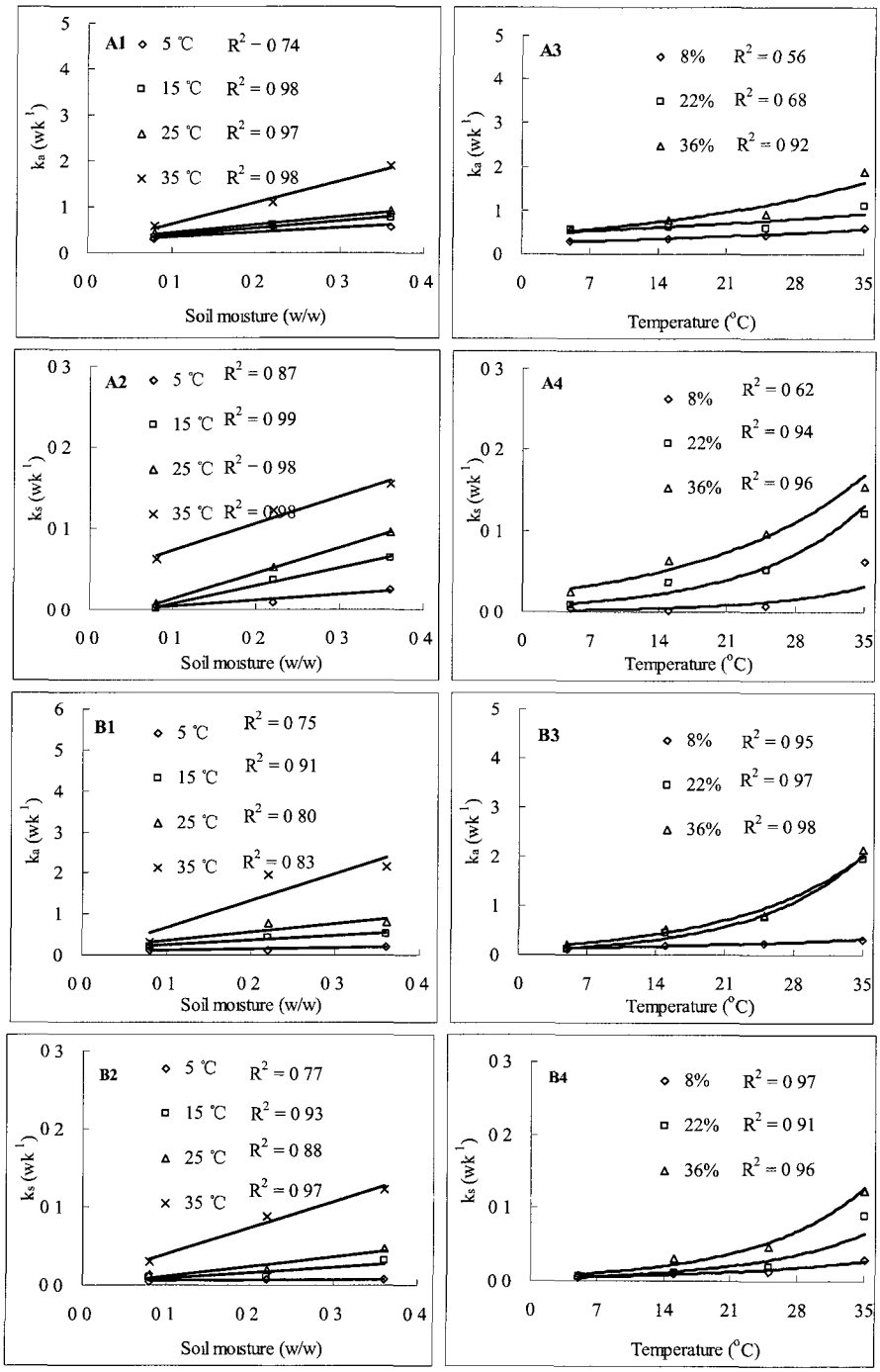

Fig. 5-1. The kinetic rate constant k_a was affected by soil moisture in soil 1 (A1) and soil 3 (A2), and by temperature in soil 3 (A3) and soil 3 (A4). The kinetic rate constant k_s was affected by soil moisture in soil 1 (B1) and soil 3 (B2), and by temperature in soil 1 (B3) and soil 7 (B4).

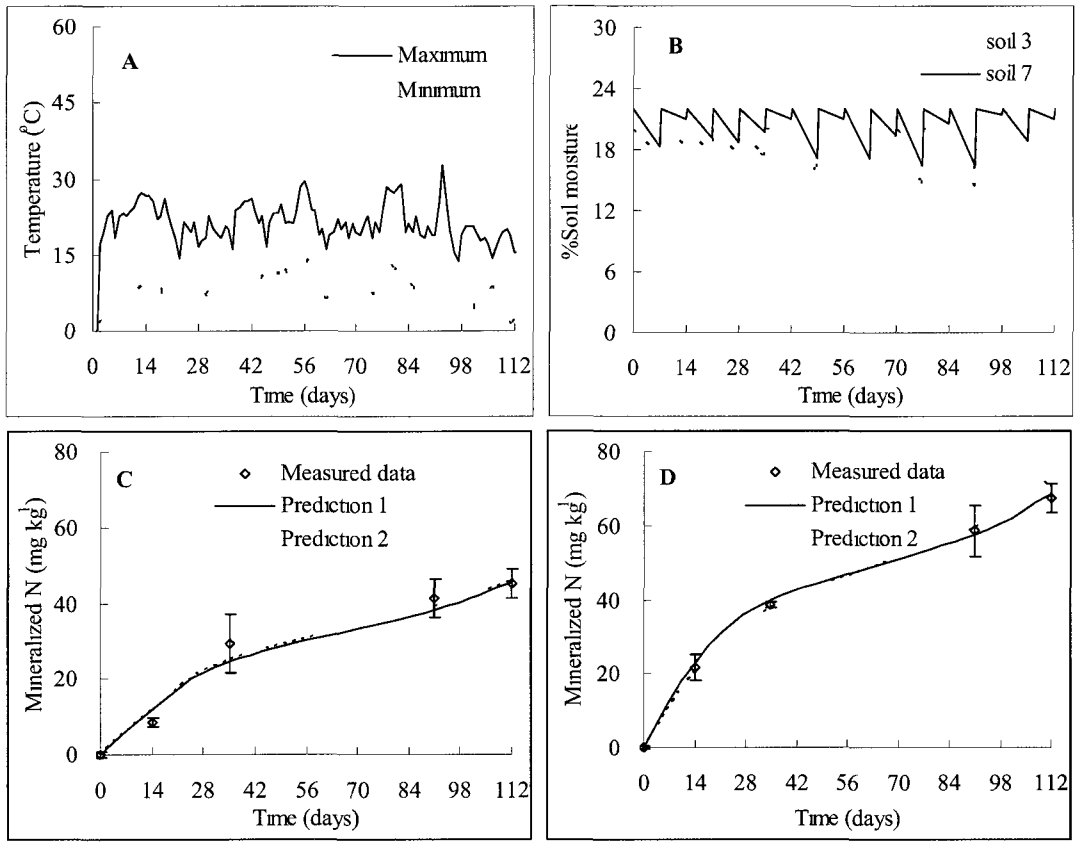

Fig 5-2. The measured and predicted (double exponential model) N mineralization for agricultural soil 3 (C) and 7 (D) during a 112-d field incubation under fluctuating temperature (A) and soil moisture (B) condition.

In prediction 1, the N_a and N_s of double exponential model were estimated from lab incubation at 15 °C and 55% water holding capacity In prediction 2, the N_a and N_s were estimated using chemical methods. Bars were standard errors of three replicates of measurements at each sampling time. Soil moisture is calculated as weight content based on water holding capacity

CHAPTER 6 SYNTHESIS

In the recent decades, much research has been done on the prediction of potentially mineralizable organic N in soil (Stanford and Smith, 1972; Quemada and Cabrera, 1995; Sharifi et al., 2007). Despite great effort, there is still a lack of a reliable method to measure potentially mineralizable organic N in soil due to soil complexity of N mineralization. An integrated and comprehensive approach was used in this research, which included kinetic models, chemical extractions, and spectroscopic properties of extracted soil organic N. In addition, field incubation was used to verify the laboratory estimated potentially mineralizable organic N. I found that exponential models used for N mineralization at the relatively higher (>20 °C) temperature were useful for the cold area such as interior Alaska. Among different models, the double exponential model with fixed rate constant was best. This model divided the potentially mineralizable organic N pool into an active mineralizable organic N pool with a rate constant of 0.694 mg N kg^{-1} $weeks^{-1}$ and a slow mineralizable organic N pool with a rate constant of 0.051 mg N kg^{-1} $weeks^{-1}$. I also found that extractions by hot deionized water (DI, 80 °C) and 1 *M* NaOH were positively correlated with the potentially mineralizable organic N. This indicated the feasibility of using chemical extraction to estimate potentially mineralizable organic N under relatively cold (15 °C) environment conditions. In addition, the statistic index of Akaike information criterion (AIC) for nonlinear model comparisons was used for comparing soil N mineralization models and suggested as a tool for model selection for soil N mineralization models in the future.

In general, chemical methods often use the total amount of extracted organic N to estimate soil potentially mineralizable organic N. However, the total chemical extraction may include multiple fractions of organic N with different biodegradability. The relative labile fraction in the extracted organic N is the most important organic N that can be used by microbes. To quantify this labile fraction, I assumed that characterizing extracted organic matter might further improve the estimation of soil mineralizable organic N pool sizes. By correlating spectroscopic properties of water extractable organic matter and model estimated organic N pool, I found that the active mineralizable organic N pool showed a higher correlation with humification index (R^2=0.89, p=0.0004) than the total amount of water extractable organic N (R^2=0.49, p=0.09). This greatly improved estimation of soil mineralizable organic N pool size.

Organic matter extracted by water at both low (22 °C) and high (80 °C) temperatures has been proposed to be labile organic matter pool (Jandl and Sollins, 1997; Sparling et al., 1998). However, it is not clear which one and why is more bioactive than the other one. This study compared the biodegradability of the extracted organic matter in cold (22 °C) and hot (80 °C) water during a 21-d solution incubation and showed that the biodegradability of hot water extractable organic matter was higher than that of cold water extractable organic matter. By analyzing spectroscopic property of cold and hot water extractable organic matter, I found that hot water extracted organic matter has lower UV absorbance at 254 and 280 nm, and lower humification index, but has higher fluorescence efficiency and protein-like components than cold water extractable organic matter. The difference of these spectroscopic properties of water extractable organic

matter at different temperature can account for their difference in biodegradability. These findings provided very important information of spectroscopic properties and biodegradability of water extractable organic matter as affected by different temperature in soils. During the fluorescence spectroscopic analysis, I found that the protein-like component was tryptophan-like fluorophore in cold WEOM but tyrosine-like fluorophore in hot WEOM, which may indicate the hydrolysis of organic C and N in hot water extraction process. The bioavailable and labile organic matter in hot water extractable organic matter may include a fraction of a product of heat hydrolysis or transformation induced by high temperature. In natural conditions, it still needs to be verified if this labile or bioavailable organic matter can be released into the soil solution. Therefore, caution needs to be taken on assessing the role of hot water extractable organic matter in soil C and N cycling.

Furthermore, mineralizable N released in field incubation with variation of soil temperature and moisture content was determined to verify the mineralizable N estimated by chemical (NaOH hydrolysable organic N), spectroscopic methods, and the double kinetic model. The results indicated that both chemical extraction together with spectroscopic methods, and the double kinetic model can successfully predict the soil N mineralization over time in the field under controlled soil moisture and fluctuating temperature. As such, this research provided a useful kinetic model and chemical method that were suitable for Alaska soils to estimate mineralizable N. However, cautions must be made since in nature soil moisture and temperature often vary beyond the range of this research. Moreover, other variables such as freeze-thaw and drying-rewetting cycles have

not been included in this research, and these cycles can have large impact on mineralizable N. Many studies have shown that drying-rewetting cycles in soil can have a significant influence on N mineralization (Sparling et al., 1995; Xiang et al., 2008). The size of mineralizable organic N pool can be replenished by crop residue, manure or others. It also can be affected by land management such as tillage and cropping rotation. Therefore, laboratory-based measurements of soil inorganic nitrogen and potentially mineralizable N need to be evaluated for its suitability and reliability as predictors of field-based indices of soil nitrogen supply under these different conditions in the future. Since N mineralization can be affected by many factors such as crop residue, tillage, and fertilization, it is possible that a chemical method that is good for N estimation under some condition will not be reliable as a predictor of N mineralization under the other conditions. For example, this study found that hot water extractable organic N can be used for the prediction of potentially mineralizable N in soils without any organic matter addition. However, it is still a question about whether hot water extractable organic N can be used to predict the capacity of N supply in soil amended with organic materials such as fish meal. Therefore, it is necessary to classify the different systems (eg. tillage, fertilizer, and crop) to assess the suitability of laboratory-based measures of soil inorganic N and potentially mineralizable N as predictors of field-based indices of soil N supply. A further modification of kinetic models with specific conditions is also needed to improve the accuracy of prediction of soil potentially labile or slow mineralizable N pools.

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